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(71) Applicants: F. HOFFMANN-LA ROCHE AG [CH/CH]; Grenzacherstrasse 124, CH-4070 Basle (CH). HYBRIDON INC. [US/US]; One Innovation Drive, Worcester, MA 01605 (US).

(72) Inventors: FRANK, Bruce, L.; 374 Simpson Road, Marlborough, MA 01752 (US). GOODCHILD, John; 150 Ruggles Street, Westborough, MA 01581 (US). HAMLIN, Henry, A., Jr.; 4 Twin Oaks Circle, R.R. 2, Box 554, Holland, MA 01521 (US). KILKUSKIE, Robert, E.; 40 Ireta Road, Shrewsbury, MA 01545 (US). ROBERTS, Noel, A.; 19 Elliswick Road, Harpenden, Herts. AL5 4TP (GB). ROBERTS, Peter, C.; 18 Concord Street, Holliston, MA 01746 (US). WALTHER, Debra, M.; Apartment 722, 285 Plantation Street, Worcester, MA 01604 (US). WOLFE, Jia, L.; 14 Boston Street, Somerville, MA 02143 (US).

(74) Agent: MEZGER, Wolfgang; Grenzacherstrasse 124, CH-4070 Basle (CH).

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(57) Abstract

The present invention discloses synthetic oligonucleotides complementary to contiguous and non-contiguous regions of the HCV RNA. Also disclosed are methods and kits for inhibiting the replication of HCV, inhibiting the expression of HCV nucleic acid and protein, and for treating HCV infections.

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Oligonucleotides specific for Hepatitis C virus

This invention relates to hepatitis C virus. More particularly, this invention relates to oligonucleotides complementary to particular regions of hepatitis C virus nucleic acid and to methods of inhibiting the expression and replication of hepatitis C virus nucleic acid and protein using these oligonucleotides.

Hepatitis C virus (HCV) is an enveloped, positive sense, single-stranded RNA virus which infects hepatocytes. HCV is the major cause of non-A, non-B, acute and chronic hepatitis (Weiner et al. (1990) Lancet 335:1-3), and has been associated with hepatocellular carcinoma (see, Dienstag et al. in Harrison's Principles of Internal Medicine, 13th Ed. (Isselbacher et al., eds.) McGraw-Hill, Inc. NY (1994) pp. 1458-1483).

The genome of HCV is a positive sense, single-stranded linear RNA of approximately 9,500 bases. The organization of this genome is similar to pestiviruses and flaviviruses, with structural proteins at the 5' end and non-structural proteins at the 3' end (reviewed by Houghton et al. (1991) Hepatol. 14:381-388). The viral RNA encodes a single polyprotein which is processed by viral and cellular proteases. HCV also contains short 5' and 3' untranslated regions (UTR). The 5' UTR is the most highly conserved region of the virus (Bukh et al. (1992) Proc. Natl. Acad. Sci. (USA) 89:4942-4946). This region has been shown to facilitate internal ribosomal entry, so that translation does not occur by ribosomal scanning from the 5' RNA cap. Instead,

ribosomes bind to internal secondary structures formed by the 5' UTR (Wang et al. (1994) J. Virol. 68:7301-7307). In addition, separate experiments have shown that HCV 5' UTR sequences can control translation of downstream sequences (Yoo et al. (1992) Virol. 191:889-899). Recently, HCV was shown to replicate in cell culture (Yoo et al. (1995) J. Virol. 69:32-38).

HCV can be transmitted by transfusion and other percutaneous routes, such as self-injection with intravenous drugs. In addition, this virus can be transmitted by occupational exposure to blood, and the likelihood of infection is increased in hemodialysis units (Dienstag et al. in Harrison's Principles of Internal Medicine (13th Ed.) (Isselbacher et al., eds.) McGraw-Hill, Inc., NY (1994) pp. 1458-14843). The risk of HCV infection is also increased in organ transplant recipients and in patients with AIDS; in all immunosuppressed groups, levels of anti-HCV antibodies may be undetectable, and a diagnosis may require testing for HCV RNA. Chronic hepatitis C occurs in as many as 20 percent of renal transplant recipients. Five to 10 years after transplantation, complications of chronic liver disease account for increased morbidity and mortality (Dienstag et al., (ibid.).

Because there is no therapy for acute viral hepatitis, and because antiviral therapy for chronic viral hepatitis is effective in only a proportion of patients, emphasis has been placed on prevention through immunization (Dienstag et al., ibid.). However, for transfusion-associated hepatitis C, the effectiveness of immunoglobulin prophylaxis has not been demonstrated consistently and is not usually recommended.

Thus, there is a need for a treatment for HCV-induced hepatitis, and for methods of controlling HCV RNA and protein expression.

New chemotherapeutic agents have been developed which are capable of modulating cellular and foreign gene expression (see, Zamecnik et al. (1978) Proc. Natl. Acad. Sci. (USA) 75:280-284). These agents, called antisense oligonucleotides, bind to target single-stranded nucleic acid molecules according to the Watson-Crick rule or to double stranded nucleic acids by the Hoogsteen rule of base pairing,

and in doing so, disrupt the function of the target by one of several mechanisms: by preventing the binding of factors required for normal transcription, splicing, or translation; by triggering the enzymatic destruction of mRNA by RNase H, or by destroying the target via reactive groups attached directly to the antisense oligonucleotide.

Improved oligonucleotides have more recently been developed that have greater efficacy in inhibiting such viruses, pathogens and selective gene expression. Some of these oligonucleotides having modifications in their internucleotide linkages have been shown to be more effective than their unmodified counterparts. For example, Agrawal et al. (Proc. Natl. Acad. Sci. (USA) (1988) 85:7079-7083) teaches that oligonucleotide phosphorothioates and certain oligonucleotide phosphoramidates are more effective at inhibiting HIV-1 than conventional phosphodiester-linked oligodeoxy-nucleotides. Agrawal et al. (Proc. Natl. Acad. Sci. (USA) (1989) 86:7790-7794) discloses the advantage of oligonucleotide phosphorothioates in inhibiting HIV-1 in early and chronically infected cells.

In addition, chimeric oligonucleotides having more than one type of internucleotide linkage within the oligonucleotide have been developed. Pederson et al. (U.S. Patent Nos. 5,149,797 and 5,220,007) discloses chimeric oligonucleotides having an oligonucleotide phosphodiester or oligonucleotide phosphorothioate core sequence flanked by nucleotide methylphosphonates or phosphoramidates. Agrawal et al. (WO 94/02498) discloses hybrid oligonucleotides having regions of deoxyribonucleotides and 2'-O-methyl-ribonucleotides.

Antisense oligonucleotides have been designed that are complementary to portions of the HCV genome. For example, oligonucleotides specific for various regions of the HCV genome have been developed (see, e.g., CA 2,104,649, WO 94/05813, WO 94/08002 and Wakita et al. (1994) J. Biol. Chem. 269:14205-14210). Unfortunately, no demonstration has been made in any reasonably predictive system that any of these oligonucleotides are capable of inhibiting the replication and expression of hepatitis C Virus.

A need still remains for the development of oligonucleotides that are capable of inhibiting the replication and expression of hepatitis C virus whose uses are accompanied by a successful prognosis, and low or no cellular toxicity.

BRIEF DESCRIPTION OF THE DRAWINGS

The objects of the present invention and the various features thereof may be more fully understood from the following description, when read together with the accompanying drawings in which:

FIG. 1 is a schematic representation of the HCV target mRNA sequence and contiguous oligonucleotides of the invention;

FIG. 2A is a diagrammatic representation of the proposed secondary structure of the HCV target mRNA sequence and one representative non-contiguous oligonucleotide of the invention;

FIG. 2B is a diagrammatic representation of the proposed secondary structure of the HCV target mRNA sequence and another representative non-contiguous oligonucleotide of the invention;

FIG. 3 is a schematic representation of the RNase H cleavage assay;

FIG. 4A is a graphic representation of HCV RNase H cleavage of Region B of HCV mRNA;

FIG. 4B is a graphic representation of HCV RNase H cleavage of Region A of HCV mRNA;

FIG. 4C is a graphic representation of HCV RNase H cleavage of Region C of HCV mRNA;

FIG. 5 is a graphic representation of RNase H cleavage of HCV mRNA stimulated by non-contiguous oligonucleotides, where (–□–) refers to results from an oligonucleotide where site 2 is on the 3' end of site 1, and (--◊--) refers to results from an oligonucleotide where site 2 is on the 5' and of site 1; X axis shows the location of 5' base of site 2 in relation to the start codon;

FIG. 6 is a graphic representation showing the effect of changing the anchor chemistry of a non-contiguous oligonucleotide of the invention on RNase H cleavage activity;

FIG. 7 is a graphic representation of RNase H cleavage of HCV mRNA in the presence of non-contiguous PS oligonucleotides competing with different concentrations of a specific non-contiguous 2' OMe oligonucleotide complementary to site 1;

FIG. 8 is a schematic representation of the HCV constructs used in various assays;

FIG. 9 is a graphic representation showing inhibition of HCVLUC in HepG2 HCVLUC cells where "–" is hcv1, SEQ ID NO:28, and "-x-" is a random 20mer (r20), at varying µM concentrations of oligonucleotide;

FIG. 10 is a graphic representation showing the inhibitory effect of different oligonucleotides of the invention (at 0.2 µM) on luciferase expression, wherein numbers within bars are the position of the 3' end of the oligonucleotide relative to the translation start site;

FIG. 11A is a phosphorimage of a ribonuclease protection assay gel showing the effect of oligonucleotides of the invention or a random 20mer on the amount of HCV-specific RNA using probe 1;

FIG. 11B is a phosphorimage of a ribonuclease protection assay gel showing the effect of oligonucleotides of the invention and a random 20mer on the amount of HCV-specific RNA using probe 2; and

FIG. 11C is a schematic representation of probes 1 and 2 used in the protection assays shown in FIGS. 11A and 11B and described in Table 4.

Antisense oligonucleotide technology provides a novel approach to the inhibition of HCV expression, and hence, to the treatment or prevention of chronic and acute hepatitis and of hepatocellular carcinoma (see generally, Agrawal (1992) Trends Biotech. 10:152; and Crooke (Proc. Am. Ass. Cancer Res. Ann. Meeting (1995) 36:655). By binding to the complementary nucleic acid sequence, antisense oligonucleotides are able to inhibit splicing and translation of RNA, and replication of genomic RNA. In this way, antisense oligonucleotides are able to inhibit protein expression.

The present invention provides oligonucleotides useful for inhibiting the replication of HCV or the expression of HCV genomic or messenger RNA or protein in a cell, and for treating HCV infection.

It has been discovered that specific oligonucleotides complementary to particular portions of the HCV genomic or messenger RNA can inhibit HCV replication or expression. This discovery has been exploited to provide synthetic oligonucleotides complementary to contiguous or non-contiguous regions of the 5' untranslated region and/or to the 5' terminal end of the RNA encoding the HCV C protein. Hence the terms "contiguous" or "non-contiguous" HCV-specific oligonucleotides.

As used herein, a "synthetic oligonucleotide" includes chemically synthesized polymers of three or up to 50 and preferably from about 5 to about 30 ribonucleotide and/or deoxyribonucleotide monomers connected together or linked by at least one, and preferably more than one, 5' to 3' internucleotide linkage.

For purposes of the invention, the term "oligonucleotide sequence that is complementary to genomic or mRNA" is intended to mean an oligonucleotide that binds to the nucleic acid sequence under physiological conditions, e.g., by Watson-Crick base pairing (interaction between oligonucleotide and single-stranded nucleic acid) or by Hoogsteen base pairing (interaction between oligonucleotide and double-stranded nucleic acid) or by any other means including in the case of a oligonucleotide binding to RNA, causing pseudoknot formation. Binding by Watson-Crick or Hoogsteen base pairing under

physiological conditions is measured as a practical matter by observing interference with the function of the nucleic acid sequence.

The invention provides in a first aspect, a synthetic oligonucleotide complementary to a portion of the 5' untranslated region of hepatitis C virus, and having a nucleotide sequence set forth in Table 1F or in the Sequence Listing as SEQ ID NO:2, 5, 6, 7, 8, 14, 15, 16, 23, 24, 26, 27, 28, 29, 31, 33, 36, 37, 47, 68, 69, 70, 71, 72, 73, 74, 75, 76, and 77, or as set forth in Tables 1A and 1B as SEQ ID NOS: 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133, or a combination thereof. The contiguous oligonucleotides are targeted to contiguous regions of the 5' UTR and coding region of HCV genomic and mRNA. For example, contiguous oligonucleotides of the invention are targeted to regions within bases 78-135 or within bases 236-263 and 303-377 (see FIG. 1).

In some embodiments, the oligonucleotides of the invention are modified. In one embodiment, these modifications include at least one internucleotide linkage selected from the group consisting of alkylphosphonate, phosphorothioate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate, phosphate triester, acetamidate, or carboxymethyl ester including combinations of such linkages, as in a chimeric oligonucleotide. In one preferred embodiment, an oligonucleotide of the invention comprises at least one phosphorothioate internucleotide linkage. In another embodiment, the oligonucleotide comprises at least one or at least two inosine residues at any position in the oligonucleotide. In another embodiment, the oligonucleotide contains one or more 5-methyl-2'-deoxycytidine residues instead of the 2'deoxycytidine.

In another modification, the oligonucleotides of the invention may also include at least one deoxyribonucleotide, at least one ribonucleotide, or a combination thereof, as in a hybrid oligonucleotide. An oligonucleotide containing at least one 2'-O-methyl ribonucleotide is one embodiment of the invention. In another

embodiment, the oligonucleotide consists of deoxyribonucleotides only. The oligonucleotides may be further modified as outlined below.

In another aspect, the present invention provides a synthetic oligonucleotide complementary to at least two non-contiguous regions of an HCV messenger or genomic RNA. Non-contiguous oligonucleotides are targeted to at least two regions of the HCV genomic RNA or mRNA which are not contiguous in a linear sense but, which may be next to each other in three dimensional space due to the secondary structure or conformation of the target molecule (FIGS. 2A and 2B). In preferred embodiments, one or both portions of the "non-contiguous" oligonucleotide is complementary to the 5' untranslated region. One portion of some non-contiguous oligonucleotides includes the same 12 bases (bases 100-111) designated the "anchor" region. The other portion of such non-contiguous oligonucleotides is variable, containing 6 to 12 bases within, e.g., bases 315-340 of HCV nucleic acid. In one embodiment, one portion which is complementary to the 5' untranslated region comprises the sequence GGGGUCCUGGAG (SEQ ID NO:47), and the other portion is complementary to a 5' region of the RNA encoding the HCV C protein. Other non-contiguous oligonucleotides of the invention may be targeted to other non-contiguous regions of HCV nucleic acid. For example, in another embodiment, the portion which is complementary to the 5' untranslated region and which functions as an anchor comprises the sequence CAACACUACUCG (bases 243-254). In preferred embodiments, the non-contiguous oligonucleotide has about 18 to about 24 nucleotides in length.

In a particular embodiment, the non-contiguous oligonucleotide which is complementary to two non-contiguous regions comprises one of the sequences as set forth in the Sequence Listing as SEQ ID NO:38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, and 67, or as set forth in Table 1C as SEQ ID NO: 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147.

In another embodiment of non-contiguous oligonucleotides of the present invention, an oligonucleotide may bind to three

proximal or non-continuous regions. These oligonucleotides are called tripartite non-contiguous oligonucleotides (see for example, Table 1D). The tripartite oligonucleotides are developed as described herein for non-contiguous oligonucleotides using non-continuous oligonucleotides (as described herein) as a 2' OMe RNA anchor with a short semi-randomized DNA sequence attached. Where this short DNA sequence can bind is detected by cleavage with RNAase H as described herein, and the specific tripartite oligonucleotide of the invention may be designed. In particular, the invention provides corresponding oligonucleotides as set forth in Table 1D under SEQ ID NOS: 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158.

In some embodiments, the non-contiguous oligonucleotides of the invention are modified in the same manner as described above oe below for the contiguous oligonucleotides.

The oligonucleotides of the present invention are for use as therapeutically active compounds, especially for use in the control or prevention of hepatitis C virus infection. In other aspects, the invention provides a pharmaceutical composition comprising at least one contiguous or non-contiguous HCV-specific oligonucleotide of the invention as described above and below, and in some embodiments, this composition includes at least two different oligonucleotides (i.e., having a different nucleotide sequence, length, and/or modification(s)). The pharmaceutical composition of some embodiments is a physical mixture of at least two, and preferably, many oligonucleotides with the same or different sequences, modifications, and/or lengths. In some embodiments, this pharmaceutical formulation also includes a physiologically or pharmaceutically acceptable carrier.

In this aspect of the invention, a therapeutic amount of a pharmaceutical composition containing HCV-specific synthetic oligonucleotides is administered to the cell for inhibiting hepatitis C virus replication or of treating hepatitis C virus infection. The HCV-specific oligonucleotides are the contiguous or non-contiguous oligonucleotides of the invention. In some preferred embodiments,

the method includes administering at least one oligonucleotide, or at least two contiguous oligonucleotides, having a sequence set forth in Table 1F or in the Sequence Listing as SEQ ID NO:2, 5, 6, 7, 8, 14, 15, 16, 17, 23, 24, 26, 27, 28, 29, 31, 33, 34, 36, 37, 47, 68, 69, 70, 71, 72, 73, 74, 75, 76, and 77 or as set forth in Tables 1A and 1B as SEQ ID NOS: 78-133, or a combination thereof. In other preferred embodiments, the method includes administering at least one non-contiguous oligonucleotide, or at least two non-contiguous oligonucleotides, having a sequence set forth in Table 2 or in the Sequence Listing as SEQ ID NO: 38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, and 67, or as set forth in Tables 1C-1E as SEQ ID NOS: 134-172, or a combination thereof. The oligonucleotides may also be used in modified form.

In all methods involving the administration of oligonucleotide(s) of the invention, at least one, and preferably two or more identical or different oligonucleotides may be administered simultaneously or sequentially as a single treatment episode in the form of separate pharmaceutical compositions.

In another aspect, the invention provides a method of detecting the presence of HCV in a sample, such as a solution or biological sample. In this method, the sample is contacted with a synthetic oligonucleotide of the invention. Hybridization of the oligonucleotide to the HCV nucleic acid is then detected if the HPV is present in the sample.

Another aspect of the invention are kits for detecting HCV in a sample. Such kits include at least one synthetic, contiguous or non-contiguous of the invention, which may have the same or different nucleotide sequence, length, and/or modification(s), and means for detecting the oligonucleotide hybridized with the nucleic acid.

As mentioned before, oligonucleotides of the invention are composed of deoxyribonucleotides, ribonucleotides, 2-O-methyl-ribonucleotides, or any combination thereof, with the 5' end of one nucleotide and the 3' end of another nucleotide being covalently

linked. These oligonucleotides are at least 6 nucleotides in length, but are preferably 12 to 50 nucleotides long, with 20 to 30mers being the most common.

These oligonucleotides can be prepared by art recognized methods. For example, nucleotides can be covalently linked using art-recognized techniques such as phosphoramidite, H-phosphonate chemistry, or methylphosphonamidate chemistry (see, e.g., Goodchild (1990) Chem. Rev. 90:543-584; Uhlmann et al. (1990) Chem. Rev. 90:543-584; Caruthers et al. (1987) Meth. Enzymol. 154:287-313; U.S. Patent 5,149,798) which can be carried out manually or by an automated synthesizer and then processed (reviewed in Agrawal et al. (1992) Trends Biotechnol. 10:152-158).

The oligonucleotides of the invention may also be modified in a number of ways without compromising their ability to hybridize to HCV genomic or messenger RNA. For example, the oligonucleotides may contain other than phosphodiester internucleotide linkages between the 5' end of one nucleotide and the 3' end of another nucleotide in which other linkage, the 5' nucleotide phosphate has been replaced with any number of chemical groups, such as a phosphorothioate. Oligonucleotides with phosphorothioate linkages can be prepared using methods well known in the field such as phosphoramidite (see, e.g., Agrawal et al. (1988) Proc. Natl. Acad. Sci. (USA) 85:7079-7083) or H-phosphonate (see, e.g., Froehler (1986) Tetrahedron Lett. 27:5575-5578) chemistry. The synthetic methods described in Bergot et al. (J. Chromatog. (1992) 559:35-42) can also be used. Examples of other chemical groups, which can be used to form an internucleotide linkage, include alkylphosphonates, phosphorodithioates, alkylphosphonothioates, phosphoramidates, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. As an example, for a combination of internucleotide linkages, US Patent No. 5,149,797 describes traditional chimeric oligonucleotides having a phosphorothioate core region interposed between methylphosphonate or phosphoramidate flanking regions. Other chimerics are "inverted" chimeric oligonucleotides comprising one or more nonionic

oligonucleotide regions (e.g alkylphosphonate and/or phosphoramidate and/or phosphotriester internucleoside linkage) flanked by one or more regions of oligonucleotide phosphorothioates. Chimerics and inverted chimerics may be synthesized as discussed in the Examples for methyl phosphonate containing oligonucleotides. These "chimerics" and "inverted chimeric" oligonucleotides are a preferred embodiment for the modification of the oligonucleotides of the present invention.

Various oligonucleotides with modified internucleotide linkages can be prepared according to known methods (see, e.g., Goodchild (1990) Bioconjugate Chem. 2:165-187; Agrawal et al. (1988) Proc. Natl. Acad. Sci. (USA) 85:7079-7083; Uhlmann et al. (1990) Chem. Rev. 90:534-583; and Agrawal et al. (1992) Trends Biotechnol. 10:152-158).

Oligonucleotides which are self-stabilized are also considered to be modified oligonucleotides useful in the methods of the invention (Tang et al. (1993) Nucleic Acids Res 20; 2729-2735). These oligonucleotides comprise two regions: a target hybridizing region; and a self-complementary region having an oligonucleotide sequence complementary to a nucleic acid sequence that is within the self-stabilized oligonucleotide. These oligonucleotides form looped structures which are believed to stabilize the 3' end against exonuclease attack while still allowing hybridization to the target. Oligonucleotides of the present invention having this structure are set forth in Table 1B as SEQ ID NOS: 131, 132 and 133.

On the other hand, examples of modifications to sugars include modifications to the 2' position of the ribose moiety which include but are not limited to 2'-O-substituted with an -O- lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl, or allyl group having 2-6 carbon atoms wherein such -O-alkyl, aryl or allyl group may be unsubstituted or may be substituted (e.g., with halo, hydroxy, trifluoromethyl, cyano, nitro acyl acyloxy, alkoxy, carboxy, carbalkoxyl, or amino groups), or with an amino, or halo group. None of these substitutions are intended to exclude the native 2'-hydroxyl group in case of ribose

or 2'-H- in the case of deoxyribose. PCT Publication No. WO 94/02498 discloses traditional hybrid oligonucleotides having regions of 2'-O-substituted ribonucleotides flanking a DNA core region.

Another form of a hybrid is an "inverted" hybrid oligonucleotide which includes an oligonucleotide comprising a 2'-O-substituted (or 2' OH, unsubstituted) RNA region which is interposed between two oligodeoxyribonucleotides regions, a structure that is inverted relative to the "traditional" hyrbid oligonucleotides. Hybrid and inverted hybrid oligonucleotides may be syntesized as described in the Examples for oligonucleotides containing 2'-0-methyl RNA. The hybrid and inverted hybrid oligonucleotides of the invention are particularly preferred due to the enhanced stability and activity over time in the presence of serum. In another embodiment the hybrid or inverted hybrid may comprise at least one n-butyl phosphoramidate or methylphosphonate linkage.

Preferably, the ribonucleotide is a 2-0-methyl ribonucleotide. In another embodiment, the oligonucleotide comprises at least one, preferably one to five 2-0-methyl ribonucleotides at the 3' end of the oligonucleotide. Moreover, the oligonucleotide may further comprise at least one, preferably one to five 2-0-methyl ribonucleotides at the 5'-end.

Other oligonucleotide structures of the invention include the so-called dumbell and nicked dumbell structures (Table 1B). Ashly and Kushlan (Biochem. (1991) 30:2927-2933) describe the synthesis of oligonucleotide dumbells including nicked dumbells. A dumbell is a double-helical stem closed off by two hairpin loops. The antisense activity of nicked dumbells (dumbell molecules with free ends) is discussed by Yamakawa et al. (Nucleosides and Nucleotides (1996) 15:519-529). These oligonucleotides structures are believed to have beneficial properties similar to those of the self-stabilized oligonucleotides described above.

In another aspect the present invention relates to contiguous and non-contiguous multiplex oligonucleotides which are designed to target a polypurine or polypyrimidine sequence by a combination of duplex and triplex formation. In some cases, the multiplex oligonucleotide of the invention may be branched by adding linkers for supporting branched moieties as is known in the art. The multiplex oligonucleotides of the invention need not be continuous and may bind to two or more proximal sites as described herein for non-contiguous oligonucleotides.

Preferred contiguous and non-contiguous multiplex oligonucleotides of the invention having SEQ ID NOS: 159-172 are shown in Table 1E. These oligonucleotides target the double strand RNA stem at -217 to -209 and the adjacent polypyrimidine sequence between -218 and -222. The hybridization of an antisense sequence to the single stranded polypyrimidine target creates a polypurine-polypyrimidine duplex that can be targeted by a triplex motif to increase the oligonucleotide binding strength. These oligonucleotides therefore provide a portion of the triplex target by duplex formation with the RNA as well as the third strand of the triple helix. The multiplex oligonucleotides as designed contain an RNase H active portion for irreversible inactivation of the target RNA. The asymmetric branching amidite (Y) (Clone Tech. Palo Alto, California) is incorporated during solid phase synthesis and hydrolyzed with hydrazine monohydrate according to the manufacturer's instructions. The branching strand is added subsequently by the same solid phase approach.

Other modifications include those which are internal or are at the end(s) of the oligonucleotide molecule and include additions to the molecule of the internucleoside phosphate linkages, such as cholesteryl, cholesterol or diamine compounds with varying numbers of carbon residues between the two amino groups, and terminal ribose, deoxyribose and phosphate modifications which cleave, or crosslink to the opposite chains or to associated enzymes or other proteins which bind to the viral genome. Other examples of modified oligonucleotides include oligonucleotides with a modified base and/or sugar such as arabinose instead of ribose, or a 3', 5'-substituted

oligonucleotide having a sugar which, at one or both its 3' and 5' positions is attached to a chemical group other than a hydroxyl or phosphate group (at its 3' or 5' position).

Additionally, oligonucleotides capped with ribose at the 3' end of the oligonucleotide may be subjected to $NaIO_4$ oxidation/reductive amination. Amination may include but is not limited to the following moieties, spermine, spermidine, Tris(2-aminoethyl) amine (TAEA), DOPE, long chain alkyl amines, crownethers, coenzyme A, NAD, sugars, peptides, dendrimers.

In another embodiment, at least one cytosine base may be modified by methylation as is known in the art, e.g., 5-methylated deoxycytosine (5-Me-dC) (see Table 1B). Such methylation may be desirable, for example, to reduce immune stimulation by the oligonucleotide if necessary.

Other modified oligonucleotides are capped with a nuclease resistance-conferring bulky substituent at their 3' and/or 5' end(s), or have a substitution in one or both nonbridging oxygens per nucleotide. Such modifications can be at some or all of the internucleoside linkages, as well as at either or both ends of the oligonucleotide and/or in the interior of the molecule (reviewed in Agrawal et al. (1992) Trends Biotechnol. 10:152-158), some non-limiting examples of capped species include 3' O-methyl, 5' O-methyl, 2' O-methyl, and any combination thereof, as shown in Table 1B

Examples of some preferred contiguous and non-contiguous oligonucleotides of the invention are listed below in Tables 1A-1E. In these Tables the internucleotide linkage is PS unless otherwise mentioned.

Most preferred, an oligonucleotide has the nucleotide sequence, sugar composition, internucleotide linkages and further modifications as set forth in Tables 1A-1F and 5 for each oligonucleotide mentioned therein.

TABLE 1A

Contiguous Oligos				
SEQ ID NO:	Oligo	Sequence	Target	Description
78	HCV-126	GCACGGTCTACG	-4 to -15	
79	HCV-126 0x6	GCACGG-tctacg	-4 to -15	N=DNA n=2'-OMe RNA
80	HCV-139	CAACACUACUCG	-76 to -87	
81	HCV-152	CAACGATCTGACCTCCGCCCG	+74 to +94	
82	HCV-153	TACTCACCGGTTCCGCAGAC	-196 to -177	
83	HCV-154	GTGTACTCACCGGTTCCGCA	-193 to -174	
84	HCV-155	GGCAATTCCGGTGTACTCAC	-183 to -164	
85	HCV-156	CCTGGCAATTCCGGTGTACT	-180 to -161	
86	HCV-157	CGTCCTGGCAATTCCGGTGT	-177 to -158	
87	HCV-158	GGTCGTCCTGGCAATTCCGG	-174 to -155	
88	HCV-159	GACCCGGTCGTCCTGGCAAT	-169 to -150	
89	HCV-160	CAAGAAAGGACCCGGTCGTC	-161 to -142	
90	HCV-161	TGATCCAAGAAAGGACCCGGT	-157 to -137	
91	HCV-162	GGTTGATCCAAGAAAGGACC	-153 to -134	
92	HCV-163	GCGGGTTGATCCAAGAAAGG	-150 to -131	
93	HCV-164	CATTGAGCGGGTTGATCCAA	-144 to -125	
94	HCV-165	AGGCATTGAGCGGGTTGATC	-141 to -122	
95	HCV-169	CATAGAGGGGCCAAGGGTAC	+240 to +259	
96	HCV-186	CCCGGGAGG	-216 to -208	
97	HCV-187	CACUAUGGCUCU	-208 to -197	
98	HCV-188	UUCCGCAGACCA	-198 to -187	
99	HCV-189	GGUCGUCCUGGC	-166 to -155	
100	HCV-190	AAAUCUCCAGGC	-125 to -114	
101	HCV-191	CGACCCAACACU	-82 to -71	
102	HCV-192	AGUACCACAAGG	-63 to -52	
103	HCV-193	CCUCCCGGG	-27 to -19	
104	HCV-196	ACGAGA	-18 to -13	
105	HCV-200	GGTTTA	+15 to +20	
106	HCV-204	TTTGAG	+20 to +25	
107	HCV-208	TTTTCT	+25 to +30	

108	HCV-212	GGCTGA	+230 to +235	
109	HCV-215	ACCCGG	+235 to +240	
110	HCV-218	AGGGTA	+240 to +245	
111	HCV-236	TTCGCGACCCAACACTACT	-67 to -85	
112	HCV-237	TTCGCGACCCAACACTAC	-67 to -84	
113	HCV-238	TTCGCGACCCAACACTA	-67 to -83	
114	HCV-239	TCGCGACCCAACACTACTC	-68 to -86	
115	HCV-240	CGCGACCCAACACTACTC	-69 to -86	
116	HCV-241	GCGACCCAACACTACTC	-70 to -86	
117	HCV-242	TT*CGCGACCCAACACTACTC	-67 to -86	*C=5-methyl-2'-deoxycytidine
117	HCV-243	TTCG*CGACCCAACACTACTC	-67 to -86	*C=5-methyl-2'-deoxycytidine
117	HCV-244	TT*CG*CGACCCAACACTACTC	-67 to -86	*C=5-methyl-2'-deoxycytidine
118	HCV-245	TTCGCIACCCAACICTACTC	-67 to -86	I=2'-deoxyinosine
119	HCV1 OX4	TTCGCGACCCAACACTacuc	-67 to -86	n=2'-OMe RNA
120	HCV1 OX3	TTCGCGACCCAACACTAcuc	-67 to -86	n=2'-OMe RNA
121	HCV1 OX2	TTCGCGACCCAACACTACuc	-67 to -86	n=2'-OMe RNA
122	HCV1 9x9	uucgcgaccCAacacuacuc	-67 to -86	n=2'-OMe RNA
123	HCV1 8X8	uucgcgacCCAAcacuacuc	-67 to -86	n=2'-OMe RNA
124	HCV1 7X7	uucgcgaCCCAACacuacuc	-67 to -86	n=2'-OMe RNA
125	HCV1 6X6	uucgcgACCCAACAcuacuc	-67 to -86	n=2'-OMe RNA
126	HCV1 11X3	ttcgcgacccaACACTActc	-67 to -86	n=2'-OMe RNA
127	HCV1 9X5	ttcgcgaccCAACACtactc	-67 to -86	n=2'-OMe RNA
128	HCV1 5X9	ttcgcGACCCAacactactc	-67 to -86	n=2'-OMe RNA
129	HCV1 3X11	ttcGCGACCcaacactactc	-67 to -86	n=2'-OMe RNA
130	HCV1 OX14	TTCGCGacccaacactactc	-67 to -86	n=2'-OMe RNA

Upper case = DNA
Lower case = 2'-OMe RNA

TABLE 1B

Looped Oligonucleotides						
SEQ ID NO:	Oligo	Sequence	Target	Loop Size	Stem Size	Description
131	HCV-1ss1	TTCGCGACC**CAACAC**TACTC-**gtgttg**	-67 to -86	5 bases	6 bp	
132	HCV-3ss1	AGTACCA**CAAG**GCCTTTCGC-**cttg**	-52 to -71	5 bases	6 bp	
133	HCV-28ss1	GCCTTTCGC**GACCC**AACACT-**gggtc**	-63 to -82	4 bases	6 bp	

Bold sequences are base paired
CAPITALS ARE ANTISENSE TO TARGET SHOWN
lower case bases are added to form the hairpin and are not complementary to RNA target

TABLE 1C

Non-contiguous Oligonucleotides					
SEQ ID NO:	Oligo	Sequence	Anchor	Target	
134	HCV-140	CAACACUACUCG-actcgcaa	-76 to -87	-37 to -30	
135	HCV-141	actcgcaa-CAACACUACUCG	-76 to -87	-37 to -30	
136	HCV-150	ggtcctggag-CAACACUACU	-76 to -85	-221 to -230	
137	HCV-151	CAACACUACU-ggtcctggag	-76 to -85	-221 to -230	
138	HCV-166	ggctct-CAACACUACUCG	-76 to -87	-206 to -211	
139	HCV-167	CAACACUACUCG-ggctct	-76 to -87	-206 to -211	
140	HCV-168	cgcaagca-CAACACUACUCG	-76 to -87	-39 to -32	
141	HCV-197	acgaga-GGGGUCCUGGAG	-219 to-230	-18 to -13	
142	HCV-201	ggttta-GGGGUCCUGGAG	-219 to -230	+15 to +20	
143	HCV-205	tttgag- GGGGUCCUGGAG	-219 to -230	+20 to +25	
144	HCV-209	ttttct-GGGGUCCUGGAG	-219 to -230	+25 to +30	
145	HCV-213	GGGGUCCUGGAG-ggctga	-219 to -230	+230 to +235	
146	HCV-216	GGGGUCCUGGAG-acccgg	-219 to -230	+235 to +240	
147	HCV-219	GGGGUCCUGGAG-agggta	-219 to -230	+240 to +245	

Upper case = 2'-OMe RNA
Lower case = DNA

TABLE 1D

Tripartite Non-contiguous Oligonucleotides					
SEQ ID NO:	Oligo	Sequence	5'-sequence target	internal sequence target	3'-sequence target
148	HCV-198	acgaga-GGGGUCCUGGAG-GCUCAU	-18 to -13	-230 to -219	+1 to +6
149	HCV-199	aggatt-GGGGUCCUGGAG-GCUCAU	+10 to +15	-230 to -219	+1 to +6
150	HCV-202	ggttta-GGGGUCCUGGAG-GCUCAU	+15 to +20	-230 to -219	+1 to +6
151	HCV-203	ggttta-GCUCAU-GGGGUCCUGGAG	+15 to +20	+1 to +6	-230 to -219
152	HCV-206	tttgag-GGGGUCCUGGAG-GCUCAU	+20 to +25	-230 to -219	+1 to +6
153	HCV-207	tttgag-GCUCAU-GGGGUCCUGGAG	+20 to +25	+1 to +6	-230 to -219
154	HCV-210	ttttct-GGGGUCCUGGAG-GCUCAU	+25 to +30	-230 to -219	+1 to +6
155	HCV-211	ttttct -GCUCAU-GGGGUCCUGGAG	+25 to +30	+1 to +6	-230 to -219
156	HCV-214	GCUCAU-GGGGUCCUGGAG-gggtga	+1 to +6	-230 to -219	+230 to +235
157	HCV-217	GCUCAU-GGGGUCCUGGAG-acccgg	+1 to +6	-230 to -219	+235 to +240
158	HCV-220	GCUCAU-GGGGUCCUGGAG-agggta	+1 to +6	-230 to -219	+240 to +245

Upper case = 2'-OMe RNA
Lower case = DNA

TABLE 1E

Contiguous and Non-contiguous Multiplex Oligonucleotides					
SEQ ID NO:	Oligo	Sequence	Duplex Target	Triplex Target (Purine Strand)	Description
159	HCV-222	CCCUCCGGGGG-tcctg	-218 to -227	-212 to -222	
160	HCV-223	GGGGG-tcctg	-218 to -227	None	
161	HCV-224	CCCUCCCCCCC-Y-(GGGGG)-tcctg	-218 to -227	-212 to -222	( )= branched triplex-forming sequence, 3'-5'
162	HCV-225	GGGGG-Y-tcctg	-218 to -227	None	
163	HCV-226	CCCUCCGGGGG-Y-(CCCCC)-tcctg	-218 to -227	-212 to -222	( )= branched triplex-forming sequence, 3'-5'
164	HCV-227	GGGGG-Y-(CCCCC)-tcctg	-218 to -227	-218 to -222	( )= branched triplex-forming sequence, 3'-5'
165	HCV-228	CCCUCCGGGGG-Y-tcctg	-218 to -227	-212 to -217	
166	HCV-229	GUCUACGAGAGGGGG-Y-(CCCCCCCUCCC)-tcctg	-218 to -227/ -18 to -9	-212 to -222	( )= branched triplex-forming sequence, 3'-5'
167	HCV-230	GUCUACGAGAGGGGG-Y-tcctg	-218 to -227/ -18 to -9	None	
168	HCV-231	GUCUACGAGAGGGGG-tcctg	-218 to -227/ -18 to -9	None	
169	HCV-232	GUCUACGAGA-Y-(CCUCCC)-ggggg	-218 to -222/ -18 to -9	-212 to -217	( )= branched triplex-forming sequence, 3'-5'

170	HCV-233	GUCUACGAGA-Y-ggggg	-218 to -222/ -18 to -9	None	
171	HCV-234	CCCGGGAGGGGGGG-Y-(CCCCCCCUCCC) -tcctg	-209 to -227	-212 to -222	( )= branched triplex-forming sequence, 3'-5'
172	HCV-235	CCCGGGAGGGGGGG-Y-tcctg	-209 to -227	None	

Upper case = 2'-OMe RNA
Lower case = DNA
Y = asymmetric branching monomer

To determine whether an oligonucleotide of the invention is capable of successfully binding to its target, several assays can be performed. One assay is an RNase H assay (Frank et al. (1993) Proc. Int. Conf. Nucleic Acid Med. Applns. 1:4.14(abstract)) which is useful when a region of at least four contiguous nucleotides of the oligonucleotide is DNA and the target is RNA. Binding of the DNA portion of the oligonucleotide (ODN) to the RNA target is identified by cleavage at that site by RNase H, as shown schematically in FIG. 3.

Using this assay, three regions of HCV mRNA were investigated as RNase H sensitive areas, and were shown to be susceptible to hybridization by members of a degenerate 20mer library, Regions A, B, and C. The assay was performed with several oligodeoxynucleotide phosphorothioate 20mers targeted to these three regions and present at a concentration of 100 nM. These oligonucleotides are set forth in Table 1F

Table 1F

Oligo	Sequence (5'->3')	Position	Base	Seq.ID No.
	A			
HCV7	GGTGCACGGTCTACGAGACC	-20 to -1	310 to 329	1
HCV16	CATGGTGCACGGTCTACGAG	-17 to +3	313 to 332	2
HCV17	GCTCATGGTGCACGGTCTAC	-14 to +6	316 to 335	3
HCV2	GTGCTCATGGTGCACGGTCT	-12 to +8	318 to 337	4
HCV18	CGTGCTCATGGTGCACGGTC	-11 to +9	319 to 338	5
HCV19	TTCGTGCTCATGGTGCACGG	-9 to +11	321 to 340	6
HCV20	GGATTCGTGCTCATGGTGCA	-6 to +14	324 to 343	7
HCV21	TTAGGATTCGTGCTCATGGT	-3 to +17	327 to 346	8
HCV8	GGTTTAGGATTCGTGCTCAT	+1 to +20	330 to 349	9
HCV22	TGAGGTTTAGGATTCGTGCT	+4 to +23	333 to 352	10
HCV23	CTTTGAGGTTTAGGATTCGT	+7 to +26	336 to 355	11
HCV10	TTCTTTGAGGTTTAGGATTC	+9 to +28	338 to 357	12
HCV9	TACGTTTGGTTTTTCTTTGA	+21 to +40	350 to 369	13
HCV11	GTTGGTGTTACGTTTGGTTT	+29 to +48	358 to 377	14
HCV128	GTCTACGAGACCTCCCGGG	-27 to -9	303 to 321	36
HCV127	GCACGGTCTACGAGACCTCC	-23 to -4	307 to 326	37
	B			
HCV38	GCACGACACTCATACTAACG	-253 to -234	77 to 96	15
HCV39	GGCTGCACGACACTCATACT	-249 to -230	81 to 100	16
HCV40	TGGAGGCTGCACGACACTCA	-245 to -226	85 to 104	17
HCV41	GTCCTGGAGGCTGCACGACA	-241 to -222	89 to 108	18
HCV42	GGGGGTCCTGGAGGCTGCAC	-237 to -218	93 to 112	19
HCV43	GAGGGGGGGTCCTGGAGGCT	-233 to -214	97 to 116	20
HCV44	CCGGGAGGGGGGGTCCTGGA	-229 to -210	101 to 120	21
HCV15	GGCTCTCCCGGGAGGGGGGG	-222 to -203	108 to 127	22
HCV45	CCACTATGGCTCTCCCGGGA	-215 to -196	115 to 134	23
	C			
HCV13	AACACTACTCGGCTAGCAGT	-77 to -96	234 to 253	24
HCV26	ACCCAACACTACTCGGCTAG	-73 to -92	238 to 257	25
HCV25	CGACCCAACACTACTCGGCT	-71 to -90	240 to 259	26
HCV24	CGCGACCCAACACTACTCGG	-69 to -88	242 to 261	27
HCV1	TTCGCGACCCAACACTACTC	-67 to -86	244 to 263	28
HCV27	CTTTCGCGACCCAACACTAC	-65 to -84	246 to 265	29
HCV28	GCCTTTCGCGACCCAACACT	-63 to -82	248 to 267	30
HCV29	AGGCCTTTCGCGACCCAACA	-61 to -80	250 to 269	31
HCV30	CAAGGCCTTTCGCGACCCAA	-59 to -78	252 to 271	32
HCV31	CACAAGGCCTTTCGCGACCC	-57 to -76	254 to 273	33
HCV32	ACCACAAGGCCTTTCGCGAC	-55 to -74	256 to 275	34
HCV3	AGTACCACAAGGCCTTTCGC	-52 to -71	259 to 278	35

TABLE 1F (continued)

Oligo	Sequence (5'->3')	Position	Base	Seq. ID No.
OTHER OLIGOS				
HCV37	CATGGCTAGACGCTTTCTGC	-274 to -255	56 to 75	69
HCV5	TGAGCGGGTTGATCCAAGAA	-128 to -147	183 to 202	71
HCV6	GATCCAAGAAAGGACCCGGT	-138 to -157	167 to 186	72
HCV14	CTCGCGGGGGCACGCCCAAA	-116 to -97	214 to 223	70
HCV12	GGCTAGCAGTCTCGCGGGGG	-106 to -87	224 to 243	73
HCV36	TTCGCGACCCAACACTACTC GGCTAGCA	-94 to -67	236 to 263	68
HCV35	GCCTTTCGCGACCCAACACT ACTCGGCT	-90 to -63	240 to 267	74
HCV34	CTTTCGCGACCCAACACTAC TCGG	-88 to -65	242 to 265	75
HCV33	CGCGACCCAACACTAC	-84 to -69	246 to 261	76
HCV4	GGGGCACTCGCAAGCACCCT	-44 to -25	285 to 304	77

Region A (or site 2) (located around the start codon) shows two peaks of activity in the RNase H cleavage assay with oligonucleotides targeted to -12 to +8 and +1 to +20 (FIG. 4B). Region B (or site 1) (located upstream at approximately bases 210-260) shows a single peak of activity that corresponds to an oligonucleotide 20mer from -237 to -218 (FIG. 4A). Region C (located upstream at bases 50-80) shows one peak of activity in this assay, for oligonucleotides targeted to -69 to -88 (FIG. 4C).

When the secondary structure of the oligonucleotides was examined, it was noted that the valley of activity between the peaks in Region A corresponds to oligonucleotides with stably folded stem-loops ($\Delta G<-2$ Kcal/mol). This suggests that secondary structure within the oligonucleotide can impede its ability to bind.

In order to determine whether the accessible sites found in the random library experiment could be used to reach other non-contiguous sites, a sequence in Region B was selected as the anchor for a semirandom oligonucleotide probe (SOP). The SOP has a defined 2'-OMe RNA "anchor" sequence complementary to

bases -219 to -230 in Region B and a six base random DNA "tail" on either its 5' or 3' end. The 2'-OMe RNA portion cannot activate RNase H cleavage and a six base random DNA library without the anchor does not activate RNase H cleavage of the transcript under these conditions. RNase H cleavage only occurs by the anchor-facilitated binding of the six-base DNA tail to the target. These semirandom oligonucleotides efficiently activate RNase H cleavage at several sites, including near the anchor, near the start codon (Region A) and within the coding region of the mRNA.

Using Region B as an anchor, Region A was targeted with non-contiguous oligonucleotide probes (NOPs). A series of NOPs were prepared that were able to bridge between Regions A and B. Maintaining the 2'-OMe anchor of the semirandomers (-219 to -230) allowed the sequence of the six base tail and the site of attachment to the anchor to be varied to find the best bridging sequence. The results of this experiment suggests that attaching the tail to different ends of the anchor gives a different optimal sequence, as shown by the different peaks of activity with RNase H. (FIG. 5).

The chemistry of the anchor of one NOP was modified to examine its effect on the binding strength of the tail. As shown in FIG. 6, modification of the 2'-OMe phosphodiester (PO) anchor to 2'-OMe phosphorothioate (PS) and DNA PS effected the cleavage efficiency of the tail. Cleavage paralleled the expected binding strength of the anchor, 2'-OMe PO > 2'-OMe PS > DNA PS.

In order to establish the necessity of anchor binding for hybridization of the tail, a competition experiment was performed. In this experiment the binding of the anchor had to compete with increasingly higher concentrations of 2'-OMe PO 12mer of the same sequence. If binding of the anchor and tail are cooperative, the cleavage by the tail should decrease as the anchor is displaced by competitor (HCV82 (SEQ ID NO:47)). As seen in FIG. 7, cleavage of RNA decreases as the concentration of competitor increases. Surprisingly, a 1000-fold excess of competitor over NOP decreases cleavage only from 46% to 20%.

This suggests that the 6 base tail imparts significant binding strength to the anchor so as to compete for Region B.

More than 40 contiguous oligonucleotide sequences were evaluated as antisense inhibitors of HCV 5, UTR-dependent protein expression (FIG. 1). Some of these oligonucleotides had different chemical backbone modifications. These oligonucleotides were evaluated in three cellular assay systems: (1) inhibition of HCV luciferase (HCVLUC) fusion protein expression in stably transfected cells; (2) inhibition of HCV RNA expression in stably transfected cells; and (3) inhibition of HCV protein expression in Semliki Forest virus/HCV recombinant virus infected cells. They were also evaluated in RNase H cleavage.

In the luciferase assay, the 5' UTR region of HCV containing the ATG start site was cloned 5' to the open reading frame of firefly luciferase (FIG. 8). Transcription of this HCV-luciferase gene fusion is stimulated in mammalian cells by a strong constitutive CMV promoter. Translation of the fusion gene is initiated at the HCV ATG which replaced the native luciferase ATG, and produces a protein which contains the first three amino acids of the viral protein and 648 amino acids of luciferase. Expression of this enzyme in mammalian cells, including the native host cells for HCV infection, can be quantified easily in a luminometer by addition of luciferin substrate and ATP cofactor to the lysed cells. Antisense oligonucleotides, when added to mammalian cells expressing this fusion construct, will reduce luciferase activity if these compounds target sequences within the 5' UTR of HCV and/or luciferase.

Both contiguous and non-contiguous oligonucleotides of the invention showed sequence specific inhibition of luciferase expression in HCVLUC cells. FIG. 9 shows a dose response for inhibition by oligonucleotide HCV1 (SEQ ID NO:28). This oligonucleotide is antisense to HCV sequences 244 to 263 (-86 to -67 relative to the start of translation for HCV) (see FIG. 1 and Table 1). Under these assay conditions, HCV1 inhibited luciferase by more than 50% at 1 and 0.2 μM relative to cells treated

without oligonucleotide. No inhibition was observed at 0.04 and 0.008 μM. In the same experiment, a random 20mer (synthesized by including all four nucleotide phosphoramidites in every step of synthesis) did not inhibit but instead enhanced luciferase at 1 μM and 0.2 μM (FIG. 9).

These results suggest that inhibition was sequence specific. Additional oligonucleotides were evaluated to extend this observation. Sense (5' -> 3'), scrambled (3' -> 5'), and mismatched oligonucleotides did not inhibit HCVLUC under conditions that HCV1 inhibited by greater than or equal to 50%. These oligonucleotides all enhanced luciferase expression at concentrations where HCV1 inhibited luciferase. These results confirm that the inhibition was highly sequence specific.

A series of oligonucleotides targeted at different sequences in the 5' UTR were evaluated in this assay system (FIG. 1). Dose response curves (1 μM to 0.008 μM) were developed for all oligonucleotide sequences. In all oligonucleotides tested, 0.2 μM was the lowest concentration which showed significant luciferase inhibition. A summary of the inhibition at 0.2 μM is shown in FIG. 10. Not all oligonucleotides targeted against HCV 5' UTR sequences inhibited luciferase expression. More active oligonucleotides (for example, HCV1 and HCV3) had percent control values less than or equal to 50 percent in these experiments. Several oligonucleotides (for example, HCV37 (SEQ ID NO:69) and HCV14 (SEQ ID NO:70) had percent control values greater than 100 percent. The most active oligonucleotides were HCV1, HCV3, and HCV28. All are targeted in the same region, HCV sequences 240 to 290. A second region, HCV sequences 80 to 140, also was complementary to oligonucleotides that inhibited luciferase.

All oligonucleotides evaluated in this assay were designed to bind to HCV sequences. Since HCVLUC created a fusion between HCV and luciferase sequences 9 bases into the coding sequence, oligonucleotides HCV8, HCV10, and HCV19-23 all had greater than 4 mismatches with the HCVLUC sequence. None of these oligonucleotides inhibited luciferase expression. These results also

confirm that sequence specific interaction with the target was required for luciferase inhibition.

Non-contiguous oligonucleotides were also evaluated in this assay. Oligonucleotides HCV53 (SEQ ID NO:39), HCV112 (SEQ ID NO:64), and HCV125 (SEQ ID NO:66), were tested and found to inhibit HCVLUC by greater than or equal to 50% at 1 μM. In addition to the anchor region, HCV53 targeted bases 324 to 329; HCV112 targeted sequences 324 to 335. This region may be particularly important for inhibition in these non-contiguous oligonucleotides.

These and other representative non-contiguous oligonucleotides of the invention are listed below in Table 2.

Table 2. Inhibition of HCVLUC by non-contiguous oligonucleotides

Oligonucleotide	chemistry	Sequence[a]	site 2 target[b]
HCV47 (SEQ ID NO:38)	2'OMePO.R6PS	GGGGUCCUGGAG-NNNNNN	
HCV53 (SEQ ID NO:39)	PS	GGGGUCCUGGAG-GACCGG	-9 to -4
HCV53 (SEQ ID NO:39)	2'OMePO/PS	*GGGGUCCUGGAG*-GACCGG	-9 to -4
HCV53 (SEQ ID NO:39)	2'OMePS/PS	*GGGGUCCUGGAG*-GACCGG	-9 to -4
HCV54 (SEQ ID NO:40)	2'OMePO/PS	GACCGG-*GGGGUCCUGGAG*	-9 to -4
HCV55 (SEQ ID NO:41)	2'OMePO/PS	*GGGGUCCUGGA*-GAGGATT	+10 to +15
HCV56 (SEQ ID NO:42)	2'OMePO/PS	AGGATT-*GGGGUCCUGGAG*	+10 to +15
HCV59 (SEQ ID NO:43)	2'OMePO/PS	*GGGGUCCUGGAG*-CATGGT	-3 to +3
HCV60 (SEQ ID NO:44)	2'OMePO/PS	CATGGT-*GGGGUCCUGGAG*	-3 to +3
HCV61 (SEQ ID NO:45)	2'OMePO/PS	*GGGGUCCUGGAG*-CGTGCT	+4 to +9
HCV62 (SEQ ID NO:46)	2'OMePO/PS	CGTGCT-*GGGGUCCUGGAG*	+4 to +9
HCV82 (SEQ ID NO:47)	2'OMePS/PS	*GGGGUCCUGGAG*	
HCV82 (SEQ ID NO:47)	PS	GGGGUCCUGGAG	
HCV82 (SEQ ID NO:47)	2'OMePO	*GGGGUCCUGGAG*	
HCV88 (SEQ ID NO:48)	PS	GGGGTCCTGGAG-CATGGTGCACGG	-9 to +3
HCV90 (SEQ ID NO:49)	2'OMePO/PS	*GGGGUCCUGGAG*-GGTGCA	-1 to -6
HCV91 (SEQ ID NO:50)	2'OMePO/PS	GGTGCA-*GGGGUCCUGGAG*	-1 to -6
HCV93 (SEQ ID NO:51)	2'OMePO/PS	*GGGGUCCUGGAG*-GCTCAT	+1 to +6
HCV94 (SEQ ID NO:52)	2'OMePS/PS	GCTCAT-*GGGGUCCUGGAG*	+1 to +6

TABLE 2 (CONTINUED)

Oligonucleotide	Chemistry	Sequence[a]	site 2 target[b]
HCV94 (SEQ ID NO:52)	PS	GCTCAT-GGGGTCCTGGAG	+1 to +6
HCV94 (SEQ ID NO:52)	2'OMePO/PS	GCTCAT-*GGGGUCCUGGAG*	+1 to +6
HCV96 (SEQ ID NO:53)	2'OMePO/PS	*GGGGUCCUGGAG*-ATTCGT	+7 to +12
HCV97 (SEQ ID NO:54)	2'OMePO/PS	ATTCGT-*GGGGUCCUGGAG*	+7 to +12
HCV99 (SEQ ID NO:55)	PS	GGGGTCCTGGAG-AGGATTCGTGCT	+4 to +15
HCV101 (SEQ ID NO:56)	2'OMePO/PS	*GGGGUCCUGGAG*-CGTGCTCATGGT	-3 to +9
HCV102 (SEQ ID NO:57)	PS	CATGGTGCACGG-GGGGTCCTGGAG	-9 to +3
HCV103 (SEQ ID NO:58)	PS	TGGATTCGTGCA-GGGGTCCTGGAG	4
HCV104 (SEQ ID NO:59)	PS	CGTGCTCATGGT-GGGGTCCTGGAG	-3 to +9
HCV106 (SEQ ID NO:60)	PS	GGGGTCCTGGAG-ATTCGTGCTCAT	+1 to +12
HCV107 (SEQ ID NO:61)	PS	ATTCGTGCTCATGGG-GTCCTGGAG	+1 to +12
HCV109 (SEQ ID NO:62)	2'OMePO/PS	*GGGGUCCUGGAG*-TGGTGCACGGTC	-11 to +1
HCV109 (SEQ ID NO:62)	PS	GGGGTCCTGGAG-TGGTGCACGGTC	-11 to +1
HCV110 (SEQ ID NO:63)	2'OMePO/PS	TGGTGCACGGTC-*GGGGUCCUGGAG*	-11 to +1
HCV110 (SEQ ID NO:63)	PS	TGGTGCACGGTC-GGGGTCCTGGAG	-11 to +1
HCV112 (SEQ ID NO:64)	2'OMePO/PS	*GGGGUCCUGGAG*-GCTCATGGTGCA	-6 to +6
HCV112 (SEQ ID NO:64)	PS	GGGGUCCUGGAG-GCTCATGGTGCA	-6 to +6
HCV113 (SEQ ID NO:65)	2'OMePO/PS	GCTCATGGTGCA-*GGGGUCCUGGAG*	-6 to +6
HCV113 (SEQ ID NO:65)	PS	GCTCATGGTGCA-GGGGUCCUGGAG	-6 to +6
HCV125 (SEQ ID NO:66)	2'OMePO/PS	*GGGGTCCTGGAG*-GCACGGTCTACG	-4 to -15

TABLE 2 (CONTINUED)

Oligonucleotide	chemistry	Sequence[a]	site 2 target[b]
HCV125 (SEQ ID NO:66)	PS	GGGGTCCTGGAG-GCACGGTCTACG	-4 to -15
HCV125 (SEQ ID NO:66)	2'OMePS/PS	*GGGGTCCTGGAG*-GCACGGTCTACG	-4 to -15
HCV125 (SEQ ID NO:66)	2'OMePO	*GGGGTCCTGGAG-GCACGGTCTACG*	-4 to -15
HCV125 (SEQ ID NO:66)	2'OMePS	*GGGGTCCTGGAG-GCACGGTCTACG*	-4 to -15
HCV134 (SEQ ID NO:67)	2'OMePO.R12PS	GGGGUCCUGGAG-NNNNNNNNNNNN[d]	

[a] Sequence in italic indicates 2' OMe modification.
[b] Site 2 orientation shows relative position. 5 indicates site 2 is at 5' end of oligonucleotide. 3 indicates that site at 3' end of oligonucleotide.
[c] Site 2 target is relative to the translation start site.
[d] N is an equimolar mixture of deoxynucleotides.

Oligonucleotides targeted at the HCV 5' untranslated region inhibited translation of a protein which was fused to the 5' untranslated region sequence. A longer HCV construct was also evaluated. This construct contained HCV sequences 52-1417, which encoded the C and E1 protein of HCV. The HCV construct was used to evaluate antisense oligonucleotide interaction with a larger HCV RNA. It was believed that this RNA secondary structure might resemble the HCV viral RNA more closely than the HCVLUC RNA. RNA levels were measured after oligonucleotide treatment to directly evaluate the interaction of oligos with their target.

Treatment of HepG2 HCV (52-1417) cells with antisense oligonucleotide decreased the amount of HCV specific RNA, as shown in FIGS. 11A and 11B. HepG2 cells which were not transfected with the HCV construct do not produce a specific, HCV related band with probe 1 (FIG. 11A). Similar experiments were conducted to show the specificity of probe 2 (FIG. 11B). FIG. 11A and 11B show that HCV1 and HCV3 decreased HCV RNA in HCV (52-1417) cells. The amounts of full length HCV RNA were quantitated on the phosphorimager and compared to untreated cells (Table 3).

TABLE 3

		% untreated [a]	
Oligonucleotide	Concentration (µM)	Probe 1	Probe 2
HCV1 (SEQ ID NO:28)	1.0 0.2 0.04	0 47 92	21 69 77
HCV3 (SEQ ID NO:35)	1.0 0.2 0.04	38 54 55	60 72 63
R20 (random)	1.0 0.2 0.04	316 454 126	254 471 125

[a] Intensity of the HCV RNA band in each oligonucleotide treated sample was compared to the intensity of the untreated sample.

Full length RNA was decreased by greater than or equal to 80% in cells treated with 1 µM HCV1. HCV1 and HCV3 decreased RNA levels by greater than 40% at concentrations greater than or equal to 0.2 µM. Random oligonucleotide increased HCV RNA by greater than 3 fold at concentrations greater than or equal to 0.2 µM. These results are consistent with the sequence specific decrease and the nonspecific increase seen in luciferase in HepG2 HCVLUC cells (see above). In cells treated with HCV1 and HCV3 at greater than or equal to 0.2 µM, lower molecular weight bands were visible. These bands corresponded to the size of RNA which would result from RNase H cleavage of the HCV RNA/HCV1 duplex (see vertical dashed line in FIG. 11C). With probe 1, the 5' side of the apparent cleavage was visible, since the lower molecular weight band was 85-90 bases less than the full length RNA for HCV1 and 70-75 bases less than full length RNA for HCV3. HCV1 and HCV3 were targeted to HCV RNA sequences 75-94 and 60-80 bases from the 3' end of the RNA/probe hybrid. With probe 2, the 3' side of the cleavage was present; the lower molecular weight band was about 10 bases less than the full length RNA for HCV1 and 30-40 bases less than full length for HCV3. HCV1 and HCV3 were targeted to sequences 6-25 and 21-40 bases from the 5' end of the RNA/probe

hybrid. Also, HCV1 and HCV3 are targeted to HCV RNA sequences 15 bases apart. The lower molecular weight bands detected on the gel were consistently about 15 bases apart.

The results from ribonuclease protection assays were consistent with specific oligonucleotide binding to target RNA. Neither probe by itself identified both cleavage products. The shorter fragments were not visible, probably because of their small size and non-specific background on the gel. Sequence specific degradation of HCV RNA confirmed the antisense activity of HCV1 and HCV3. The presence of cleavage products suggests that RNase H contributed to the activity of these phosphorothioate oligonucleotides in this assay system.

To confirm this observation, oligonucleotide specific RNA cleavage in cells was compared to in vitro cleavage of RNA/oligonucleotide hybrids by RNase H. HCV RNA was transcribed in vitro with T7 RNA polymerase and incubated with specific oligonucleotides and RNase H. RNA was then precipitated, and ribonuclease protection assays performed. Assays were performed as described above except that 0.1 ng in vitro transcribed RNA was used in the ribonuclease protection assay. Molecular weights of bands were determined by comparison to RNA standards.

As with oligonucleotide treated cells, specific lower molecular weight products were detected after in vitro RNase H cleavage of oligonucleotide/RNA hybrids. Molecular weights were consistent with predicted oligonucleotide binding sites and also with products detected in cells, as shown in Table 4.

TABLE 4

Size comparison of in vitro and cellular RNA treated with oligonucleotides

Unit	HCV1 (SEQ ID NO:28)	HCV3 (SEQ ID NO:35)	HCV8 (SEQ ID NO:10)
probe 1			
predicted product[a]	75-94	60-79	8
in vitro product[b]	93-97	71-80	7
cellular product[c]	87-95	72-78	6
probe 2			
predicted product[a]	6-25	21-40	92-111
in vitro product[b]	13-17	21-30	90-100
cellular product[c]	10-30	30-40	n.d.

[a] Predicted product is the molecular weight difference between the full length RNA and the RNA remaining after oligonucleotide binding and RNase cleavage.

[b] In vitro product is the molecular weight difference between full length RNA and RNA detected after in vitro RNase H cleavage in the presence of oligonucleotide.

[c] Cellular product is the molecular weight difference between full length RNA and RNA detected after treatment of target containing cells with oligonucleotide.

With probe 1 (FIG. 11C), HCV1 produced bands 90-95 bases less than full length RNA; HCV3 produced bands 70-80 bases less than full length RNA. With probe 2 (FIG. 11C), products were 13-17 bases less than full length for HCV1, 20-30 bases less than full length for HCV3. In summary, these results show that oligonucleotides inhibited RNA

production by sequence-specific interaction with target RNA, and subsequent degradation by cellular RNase H.

SFV/HCV recombinant virus was prepared as a model system for measuring HCV protein production after virus infection. pSFV1/HCV (containing HCV sequence 1-2545) was prepared from a plasmid (Hoffman-Roche, Basel, Switzerland) and pSFV1 (Gibco/BRL, Gaithersburg, MD). RNA transcribed from pSFV1/HCV produces SFV replicase proteins which replicate the input RNA and produce multiple copies of subgenomic mRNA. The subgenomic RNA contains the 5' end of HCV RNA plus approximately 50 bases derived from the pSFV1 vector. This model has the advantages of cytoplasmic replication and a 5' end very similar to authentic HCV.

Recombinant SFV/HCV infected three cell types: HepG2; CHO; and BHK21. Infection was monitored by HCV C protein production. Cells were infected for 1 hour, inoculum was removed, and cells were cultured overnight. Cells were lysed and protein separated on a 13.3% polyacrylamide/SDS gel. Proteins were electroblotted onto nitrocellulose and detected by Western blot using rabbit anti-HCV C protein antiserum. Protein was detected after infection with a 1/750 virus dilution in HepG2 and CHO cells and 1/3750 virus dilution in BHK21 cells. Antisense experiments were conducted in HepG2 cells using a 1/100 virus dilution.

HCV C protein was decreased in the presence of HCV1. The inhibition was 50% at 2 μM and 0.4 μM HCV1. No consistent decrease was detected in randomer treated cells.

Additional oligonucleotides were also evaluated in this assay. HCV3 inhibited C protein production by about 60 to 70% at 0.4 μM; and HCV8 inhibited C protein production by about 40% at 2 μM and 0.4 μM.

In summary, the SFV/HCV recombinant provided a model system for HCV replication, and in a sequence specific inhibition of HCV protein expression was measured.

Some modified oligonucleotides were evaluated as luciferase inhibitors in HepG2 HCVLUC cells. Experiments were conducted with phosphorothioate oligodeoxynucleotides and with oligonucleotides having additional backbone modifications (chimeric and hybrid). In addition, the effects of oligonucleotide length on activity of modified backbones were also evaluated. The results of these experiments are shown in Table 5 below.

TABLE 5

Oligonucleotide	Sequence	Modification	HepG2 HCVLUC (% control) at 0.2 μM[a])
HCV1 (SEQ ID NO:28)	244-263	PS	46±18
EG4-7 (SEQ ID NO:28)	244-263	5'-(PO,2'OMe)$_{20}$-3'	100
EG4-10 (SEQ ID NO:28)	244-263	5'-(PO)$_{15}$(PO,2'OMe)$_5$-3'	99
EG4-13 (SEQ ID NO:28)	244-263	5'-(PO,2'OMe)$_5$-(PO)$_{10}$(PO,2'OMe)$_5$-3'	86±2
EG4-17 (SEQ ID NO:28)	244-263	5'-(PS,2'OMe)$_{20}$-3'	129±64
EG4-20 (SEQ ID NO:28)	244-263	5'-(PS)$_{15}$- (PS,2'OMe)$_5$-3'	48±27
EG4-23 (SEQ ID NO:28)	244-263	5'-(PS,2'OMe)$_5$-(PS)$_{10}$(PS,2'OMe)$_5$-3'	57±20
EG4-29 (SEQ ID NO:28)	244-263	5'-(PS)$_{15}$(PO,2'OMe)$_5$-3'	67±13
EG-4-65 (SEQ ID NO:28)	244-263	5'-(PO,2'OMe)$_5$-(PO)$_{10}$(PO,2'OMe)$_5$-3'	82±11

[a] - average ± standard deviation
[b] - number of experiments

Hybrid oligonucleotides having SEQ ID NO:28 and having residues containing 2' OMe RNA at the 3' end or both ends, inhibited luciferase.

The most active modifications were five 2'OMe RNA phosphorothioate residues at the 3'end (EG4-20) or five 2'OMe RNA phosphorothioate residues at both ends (EG4-23). An oligonucleotide containing all 2'OMe phosphorothioate residues (EG4-17) did not inhibit luciferase. This suggests that RNase H is necessary for luciferase inhibition since 2'OMe residues are not substrates for RNase H. Hybrid oligonucleotides containing five 2'OMe phosphodiester residues at the 3' end (EG4-29) or five 2'OMe phosphodiester residues at both ends (EG4-65) were less active than their phosphorothioate counterparts. This suggests that phosphorothioate linkages are required for maximum activity.

Chimeric oligonucleotides can be prepared which contained phosphoramidate or methylphosphonate linkages in addition to phosphorothioate linkages. All sequences were based on HCV36 (SEQ ID NO:68) or HCV25 (SEQ ID NO:26). The results of luciferase inhibition studies using oligonucleotides having phosphorothioate and methylphosphonate linkages are shown below in Table 6.

TABLE 6

Compound	Sequence	SEQ ID No.	Modification	HepG2 HCVLUC (%control) at 0.2μM[a])
HCV36	236-263	68	PS	56
HCF36M	236-263	68	5'-$(PS)_{22}(PM)_5$-3'[a]	54
HCV36M2	236 -263	68	5'-$(PS)_2PM(PS)_8PM$ $(PS)_9PM(PS)_6PMPS$-3'	73
HCV36M3	236-263	68	5'-$(PS)_2PM_2(PS)_7PM$ $(PS)_9PM(PS)_6(PM)_2PS$-3'	62
HCV25	240-259	26	PS	42
HCV25M	240-259	26	5'-$(PS)_{14}(PM)_5$-3	75

a PM = P-Methyl

In summary, antisense activity, as measured by luciferase inhibition, was retained in molecules with several backbone modifications: (1) oligonucleotides with phosphorothioate internucleotide linkages, (2) hybrids with DNA phosphorothioate internucleotide linkages and 2'-O-methyl RNA; (3) chimeric oligonucleotides having phosphorothioate and methylphosphonate internucleotide linkages. Chimeric oligonucleotides having phosphorothioate and PNBu internucleotide linkages and chimeric oligonucleotides having phosphorothioate and $PNH(CH_2)_6NH^{3+}$ internucleotide linkages should also be effective. Antisense activity appeared to require phosphorothioate rather than phosphodiester backbones; longer chain lengths with chimeric oligonucleotides (that hybridize less strongly); and the ability to activate ribonuclease H.

The synthetic antisense oligonucleotides of the invention in the form of a therapeutic composition or formulation are useful in inhibiting HCV replication in a cell, and in treating hepatitis C viral infections and resulting conditions in an animal, such as chronic and acute hepatitis, hepatocellular carcinoma. They may be used on or as part of a pharmaceutical composition when combined with a physiologically and/or pharmaceutically acceptable carrier. The characteristics of the carrier will depend on the route of administration. Such a composition may contain, in addition to the synthetic oligonucleotide and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which enhance inhibition of HCV expression. For example, combinations of synthetic oligonucleotides, each of which is directed to different regions of the HCV genomic or messenger RNA, may be used in the pharmaceutical compositions of the invention. The pharmaceutical composition of the invention may further contain other chemotherapeutic drugs. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with the synthetic oligonucleotide of the invention, or to minimize side-effects caused by the synthetic oligonucleotide of the invention. Conversely, the synthetic oligonucleotide of the invention may be included in formulations of a

particular anti-HCV or anti-cancer factor and/or agent to minimize side effects of the anti-HCV factor and/or agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which the synthetic oligonucleotides of the invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which are in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323. The pharmaceutical composition of the invention may further include compounds such as cyclodextrins and the like which enhance delivery of oligonucleotides into cells , or such as slow release polymers.

As used herein, the term "therapeutically effective amount" or "therapeutic amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., reduction in chronic or acute hepatitis or hepatocellular carcinoma. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of one or more of the synthetic oligonucleotides of the invention is administered to a subject afflicted with an HCV-associated disease. The synthetic oligonucleotide of the invention may be administered in accordance with the method of the invention either alone of in combination with other known therapies for the HCV-associated disease. When co-administered with one or more other therapies, the synthetic oligonucleotide of the

invention may be administered either simultaneously with the other treatment(s), or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering the synthetic oligonucleotide of the invention in combination with the other therapy.

Administration of the synthetic oligonucleotide of the invention used in the pharmaceutical composition or to practice the method of treating an animal can be carried out in a variety of conventional ways, such as intraocular, oral ingestion, inhalation, or cutaneous, subcutaneous, intramuscular, or intravenous injection.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered orally, the synthetic oligonucleotide will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% synthetic oligonucleotide and preferably from about 25 to 90% synthetic oligonucleotide. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the synthetic oligonucleotide and preferably from about 1 to 50% synthetic oligonucleotide.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered by intravenous, cutaneous or subcutaneous injection, the synthetic oligonucleotide will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in

addition to the synthetic oligonucleotide, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 1.0 ng to about 2.5 mg of synthetic oligonucleotide per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the synthetic oligonucleotide will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

The invention also provides kits for inhibiting hepatitis C virus replication and infection in a cell. Such a kit includes a synthetic oligonucleotide specific for HCV genomic or messenger RNA, such as those described herein. For example, the kit may include at least one of the synthetic contiguous oligonucleotides of the invention, such as

those having SEQ ID NO: 2, 5, 6, 7, 8, 14, 15, 16, 23, 24, 26, 27, 28, 29, 31, 33, 36, 37, 47, and/or at least one of the non-contiguous oligonucleotides having SEQ ID NO: 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, and 67 and/or those oligonucleotides having SEQ ID NOS: 78-172 and listed in Tables 1A-1E. These oligonucleotides may have modified backbones, such as those described above, and may be RNA/DNA hybrids containing, for example, at least one 2'-O-methyl. The kit of the invention may optionally include buffers, cell or tissue preparation reagents, cell or tissue preparation tools, vials, and the like.

In another aspect, the invention provides a method of detecting the presence of HCV in a sample, such as a solution or biological sample. In this method, the sample is contacted with a synthetic oligonucleotide of the invention. Hybridization of the oligonucleotide to the HCV nucleic acid is then detected if the HCV is present in the sample.

Another aspect of the invention are kits for detecting HCV in a sample. Such kits include a contiguous or non-contiguous synthetic oligonucleotide of the invention, and means for detecting the oligonucleotide hybridized with the nucleic acid.

The following examples illustrate the preferred modes of making and practicing the present invention, but are not meant to limit the scope of the invention since alternative methods may be utilized to obtain similar results.

EXAMPLES

1. Oligonucleotide Synthesis

Oligonucleotides were synthesized using standard phosphoramidite chemistry (Beaucage (1993) Meth. Mol. Biol. 20:33-61; Uhlmann et al. (1990) Chem. Rev. 90:543-584) on either an ABI 394 DNA/RNA synthesizer (Perkin-Elmer, Foster City, CA), a Pharmacia Gene Assembler Plus (Pharmacia, Uppsala, Sweden) or a

Gene Assembler Special (Pharmacia, Uppsala, Sweden) using the manufacturers standard protocols and custom methods. The custom methods served to increase the coupling time from 1.5 min to 12 min for the 2'-OMe RNA amidites. The Pharmacia synthesizers required additional drying of the amidites, activating reagent and acetonitrile. This was achieved by the addition of 3 Å molecular sieves (EM Science, Gibbstown, NJ) before installation on the machine.

DNA ß-cyanoethyl phosphoramidites were purchased from Cruachem (Glasgow, Scotland). The DNA support was 500 Å pore size controlled pore glass (CPG) (PerSeptive Biosystems, Cambridge, MA) derivatized with the appropriate 3' base with a loading of between 30 to 40 mmole per gram. 2'-OMe RNA ß-cyanoethyl phosphoramidites and CPG supports (500 A) were purchased from Glen Research (Sterling, VA). For synthesis of random sequences, the DNA phosphoramidites were mixed by the synthesizer according to the manufacturer's protocol (Pharmacia, Uppsala, Sweden).

All 2'-OMe RNA-containing oligonucleotides were synthesized using ethylthiotetrazole (American International Chemical (AIC), Natick, MA) as the activating agent, dissolved to 0.25 M with low water acetonitrile (Aldrich, Milwaukee, WI). Some of the DNA-only syntheses were done using 0.25 M ethylthiotetrazole, but most were done using 0.5 M 1-H-tetrazole (AIC). The thiosulfonating reagent used in all the PS oligonucleotides was 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage Reagent) (R.I. Chemical, Orange, CA, or AIC, Natick, MA) as a 2% solution in low water acetonitrile (w/v).

After completion of synthesis, the CPG was air dried and transferred to a 2 ml screw-cap microfuge tube. The oligonucleotide was deprotected and cleaved from the CPG with 2 ml ammonium hydroxide (25-30%). The tube was capped and incubated at room temperature for greater than 20 minutes, then incubated at 55°C for greater than 7 hours. After deprotection was completed, the tubes were removed from the heat block and allowed to cool to room temperature. The caps were removed and the tubes were microcentrifuged at 10,000 rpm for 30 minutes to remove most of the ammonium hydroxide. The liquid was then transferred to a new 2 ml

screw cap microcentrifuge tube and lyophilized on a Savant speed vac (Savant, Farmingdale, NY). After drying, the residue was dissolved in 400 µl of 0.3 M NaCl and the DNA was precipitated with 1.6 ml of absolute EtOH. The DNA was pelleted by centrifugation at 14,000 rpm for 15 minutes, the supernatant decanted, and the pellet dried. The DNA was precipitated again from 0.1 M NaCl as described above. The final pellet was dissolved in 500 µl H_2O and centrifuged at 14,000 rpm for 10 minutes to remove any solid material. The supernatant was transferred to another microcentrifuge tube and the amount of DNA was determined spectrophotometrically. The concentration was determined by the optical density at 260 nM. The E_{260} for the DNA portion of the oligonucleotide was calculated by using OLIGSOL (Lautenberger (1991) Biotechniques 10:778-780). The E_{260} of the 2'-OMe portion was calculated by using OLIGO 4.0 Primer Extension Software (NBI, Plymouth, MN).

Oligonucleotide purity was checked by polyacrylamide gel electrophoresis (PAGE) and UV shadowing. 0.2 OD_{260} units were loaded with 95% formamide/H_2O and Orange G dye onto a 20% denaturing polyacrylamide gel (20 cm x 20 cm). The gel was run until the Orange G dye was within one inch of the bottom of the gel. The band was visualized by shadowing with shortwave UV light on a Keiselgel 60 F254 thin layer chromatography plate (EM Separations, Gibbstown, NJ).

2. <u>Synthesis and Purification of Oligonucleotides Containing Mixed Backbones</u>

Standard phosphoramidite chemistry was applied in the synthesis of oligonucleotides containing methylphosphonate linkages using two Pharmacia Gene Assembler Special DNA synthesizers. One synthesizer was used for the synthesis of phosphorothioate portions of oligonucleotides using β-cyanoethyl phosphoramidites method discussed above. The other synthesizer was used for introduction of methylphosphonate portions. Reagents and synthesis cycles that had been shown advantageous in methylphosphonate synthesis were applied (Hogrefe et al., in Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs (Agrawal, ed.) (1993)

Humana Press Inc., Totowa, NJ). For example, 0.1 M methyl phosphonamidites (Glen Research) were activated by 0.25 M ethylthiotetrazole; 12 minute coupling time was used; oxidation with iodine (0.1 M) in tetrahydrofuran/2,6-lutidine/water (74.75/25/0.25) was applied immediately after coupling step; dimethylaminopyridine (DMAP) was used for capping procedure to replace standard N-methylimidazole (NMI). The chemicals were purchased from Aldrich (Milwaukee, WI).

The work up procedure was based on a published procedure (Hogrefe et al. (1993) Nucleic Acids Research 21:2031-2038). The product was cleaved from the resin by incubation with 1 ml of ethanol/acetonitrile/ammonia hydroxide (45/45/10) for 30 minutes at room temperature. Ethylenediamine (1.0 ml) was then added to the mixture to deprotect at room temperature for 4.5 hours. The resulting solution and two washes of the resin with 1 ml 50/50 acetonitrile/0.1 M triethylammonium bicarbonate (TEAB), pH 8, were pooled and mixed well. The resulting mixture was cooled on ice and neutralized to pH 7 with 6 N HCl in 20/80 acetonitrile/water (4-5 ml), then concentrated to dryness using the Speed Vac concentrater. The resulting solid residue was dissolved in 20 ml of water, and the sample desalted by using a Sep-Pak cartridge. After passing the aqueous solution through the cartridge twice at a rate of 2 ml per minute, the cartridge was washed with 20 ml 0.1 M TEAB and the product eluted with 4 ml 50% acetonitrile in 0.1 M TEAB at 2 ml per minute. The eluate was evaporated to dryness by Speed Vac. The crude product was purified by the PAGE procedure, desalted using a Sep-Pak cartridge, then exchanged counter ion into sodium by ethanol precipitation of NaCl solutions, as described above. The product was dissolved in 400 ml water and quantified by UV absorbance at 260 nM.

3. <u>Constructs</u>

The oligonucleotide constructs which were used are shown schematically in FIG. 9. The HCV-luciferase fusion protein (HCVLUC) contained bases 52 to 338 of HCV sequence. HCV sequences 52-337 (Kato et al. (1990) Proc. Natl. Acad. Sci. (USA) 87:9524) were

subcloned from plasmid pHO3-65 (Hoffmann-La Roche, Basel, Switzerland) using PCR. The 5' primer was a T7 primer which is upstream of the HCV region in pHO3-65. The 3' PCR primer contained bases complementary to luciferase and 18 bases complementary to HCV. The PCR product was subcloned into pCRII (Invitrogen, San Diego, CA). The correct sequence confirmed and then cloned into pGEMluc (Promega, Madison, WI). This fused HCV sequences to luciferase, substituting the first 9 bases of HCV for the first 6 bases of luciferase to make pGEMHCVLUC. HCVLUC sequences were subcloned into pcDNAIneo (Invitrogen, San Diego, CA) to produce pcHCVLUCneo for stable expression in mammalian cells.

HCV sequences 52-337 and 254-1417 (Kato et al. (1990) Proc. Natl. Acad. Sci. (USA) 87:9524) from pHO3-65 and pHO3-62 (Hoffmann-La Roche, Basel, Switzerland), respectively, were subcloned together into pBluescriptIISK (Stratagene, La Jolla, CA) to produce HCV sequences 52-1417 in a single vector. HCV52-1417 was then subcloned into pcDNAIneo (Invitrogen, San Diego, CA) to produce pcHCVneo.

4. RNase H Assays

A. Plasmid Preparation

The pcHCVneo plasmid (10 μg) was linearized with XbaI restriction enzyme (New England Biolabs, Beverly, MA, 20 U) for 2 hours at 37°C, treated with proteinase K (Stratagene, La Jolla, CA) (0.1 μg/μl) for 1 hour at 37°C and twice phenol/chloroform extracted. The linearized plasmid was ethanol precipitated and isolated from the supernatant by centrifugation. The dried pellet was dissolved in diethylpyrocarbonate (DRPC) (Aldrich, Milwaukee, WI)-treated water to a concentration of 0.5 μg/μl.

B. In Vitro Transcription and ^{32}P-Labelling of HCV mRNA

HCV mRNA was transcribed in vitro using either the Stratagene mRNA Transcription Kit (La Jolla, CA) or the Ambion MEGAscript In vitro Transcription Kit (Austin, TX), and each manufacturers T7 RNA

polymerase supplied with each kit. Transcription was performed in the presence of 7.5 mM CTP, 7.5 mM ATP, 75 mM UTP, 6 mM GTP, and 6 mM guanosine hydrate. The reduced GTP concentration allowed the initiation of a high percentage of the transcripts with guanosine to facilitate end-labelling of the mRNA without pretreatment with alkaline phosphatase. After transcribing for 3 hours at 37ϒC, the reaction was treated with RNase-free DNase (Stratagene, La Jolla, CA or Ambion, Austin, TX), twice phenol/chloroform extracted, and chromatographed through a G-50 Sephadex spin-column (Boehringer-Mannheim, Indianapolis, IN or Pharmacia, Uppsala, Sweden) to remove unreacted nucleotides and nucleoside. The recovered mRNA was quantitated by measuring the UV absorbance at 260 nm using an extinction coefficient of 10000 M^{-1} cm^{-1} $base^{-1}$ of the mRNA.

Yields were generally 200-250 µg RNA/µg DNA from a 20 µl reaction. The mRNA was aliquotted (15 µg) and stored at -80°C until needed. The mRNA (15 µg) was end-labelled with 20-25 units of T4 polynucleotide kinase (Pharmacia, Uppsala, Sweden) and 50 µCi [γ-^{32}P]ATP (Amersham, Arlington Heights, IL), 6000 Ci/mmol). The labelled mRNA was purified by chromatography through a G-50 Sephadex spin column (Boehringer-Mannheim, Indianapolis, IN, or Pharmacia, Uppsala, Sweden).

C RNase H Cleavage with Random 20mer Library

End-labelled RNA (20-100 nM) was incubated with a 20 base random DNA library (50-100 µM) (synthesized on Pharmacia Gene Assembler; all oligonucleotide synthesis, above), boiled previously to dissociate any aggregates, for 90 min at 37°C in 9 µl 1x buffer (40 mM Tris-HCl pH 7.4, 4 mM $MgCl_2$, 1 mM DTT). RNase H (Boehringer-Mannheim, Indianapolis, IN) (1 µl, 1 unit/µl) was then added. The reaction was incubated at 37°C for 10 min, quenched by addition of 10 µl 90% formamide containing 0.1% phenol red/0.1% xylene cyanol, and frozen on dry ice. The quenched reactions were boiled for 2.5 to 3 minutes, quenched on ice, and 5 to 7 µl loaded onto a denaturing 4% polyacrylamide gel prerun to 50 to 55°C. The phenol red was typically run to the bottom of the gel, which was then dried at 80°C under vacuum. The gel was autoradiographed using XOMAT film (Kodak,

Rochester, NY) or analyzed using phosphorimage technology on a Molecular Dynamics (Sunnyvale, CA) or Bio Rad Phosphorimager (Hercules, CA).

D. Cleavage of HCV mRNA with Specific Antisense Oligonucleotides

In 9 µl 1x RNase H buffer (40 mM Tris-HCl pH 7.4, 4 mM $MgCl_2$, 1 mM DTT), 20-100 nM [5'-^{32}P]-labelled mRNA and 100 nM oligonucleotides (ODN) were preincubated for 15 min at 37°C. 1 µl RNase H (1 U/µl) was added, and the reaction was incubated at 37°C for 10 min. The reactions were quenched and analyzed as described above. Quantitation of the cleavage products was performed using software supplied with the PhosphorImager (Molecular Dynamics, Sunnyvale, CA, or Bio-Rad Laboratories, Hercules, CA). "Counts" were determined by drawing a box around the band of interest and subtracting the background determined with a box drawn nearby. Counts in a product band were compared to total counts in the lane above that band to determine % cleavage. This accounts for the cleavage of small amounts of incomplete transcripts.

E. Cleavage of HCV mRNA with Semirandom Oligonucleotides

Semirandom oligonucleotides (100 µM in H_20) were boiled for 1 min to dissociate any aggregates formed between complementary sequences in the mix and 1 µl (final concentration 10 µM) was added to 8 µl 1x RNase H buffer (40 mM Tris-HCl pH 7.4, 4 mM $MgCl_2$, 1 mM DTT) containing labelled mRNA (20-100 nM). After a 15 minute preincubation at 37°C, RNase H was added (1 U) and incubated for 10 min at 37°C. The reactions were quenched and analyzed as described above. Sites of cleavage were estimated using DNA and/or RNA molecular size markers.

5. Inhibition of HCV-Luciferase Fusion Protein Expression in Stably Transfected Cells

A. Transfection

HepG2 cells (ATCC HB8065, American Type Culture Collection, Rockville, MD) were maintained in DMEM with 10% fetal calf serum. Cells were transfected with pcHCVLUCneo by the calcium phosphate procedure (Sambrook et al. (1989) Molecular Cloning, A Laboratory Manual (2nd ed.), Cold Spring Harbor Laboratory Press, pp. 16.30-16.40). Stably transfected clones were selected with (0.75 μg/ml) Geneticin (Gibco/BRL, Gaithersburg, MD). Clones were evaluated for luciferase expression as described below. A similar luciferase construct lacking HCV sequence was also expressed stably in HepG2 cells.

Cells were incubated in lysis buffer (Analytical Luminescence Laboratory, San Diego, CA). Cell lysate (20 μl) was transferred to a White Microlite Plate (Dynatech Laboratories, Chantilly, VA) and 50 μl substrate A (Analytical Luminescence Laboratory, San Diego, CA) was added to the plate. Luciferase activity was measured in a Microplate Luminometer LB96P (EG&G Berthold, Nashua, NH) by injecting 50 μl Substrate B (Analytical Luminescence Laboratory, San Diego, CA)), waiting 2 seconds, and then integrating the luminescence signal over 10 seconds.

B. Inhibition of HCVLUC Expression

HepG2 HCVLUC cells were seeded onto a 96 well plate (5000 cells/well), and incubated overnight at 37°C. Oligonucleotides were diluted in Optimem (Gibco/BRL, Gaithersburg, MD) containing 10 μg/ml Lipofectin (Gibco/BRL, Gaithersburg, MD). Medium was removed from cells and replaced with 100 μl oligonucleotide in Optimem/Lipofectin. Cells were incubated overnight, washed twice with PBS, and then luciferase expression was evaluated.

Alternatively, stably transfected HepG2 cells were treated with oligonucleotides as described previously, except that oligonucleotides were mixed with 4ug/ml Cellfectin (Gibco-BRL). Inhibition was measured at four oligonucleotide concentrations, relative to cells treated only with Cellfectin. EC_{50} was determined from graphs of the dose response curves. Most active compounds contained 5x5 and 6x6 2'OMe. When more than 12 2'OMe residues were present,

oligonucleotides were less active. In this assay, when 18 or 20 2'OMe residues were present (9x) or all 2'OMe) HCVLUC was not inhibitied at any concentration tested (up to 1 uM). The results are shown below in Table 7.

Table 7

Sequence	SEQ ID No.	Backbone	EC_{50} µM
HCV1	28	PS	0.04
HCV1	28	5x5 2'OMe PS	0.02
HCV1	28	6x6 2'OMe PS	0.03
HCV1	28	7x7 2'OMe PS	0.09
HCV1	28	8x8 2'OMe PS	0.07
HCV1	28	9x5 2'OMe PS	0.08
HCV1	28	5x9 2'OMe PS	0.05
HCV1	28	3x11 2'OMe PS	0.09
HCV1	28	11x3 2'OMe PS	0.2
HCV1	28	0x14 2'OMe PS	0.4

All oligonucleotide-treated samples were measured in triplicate wells. Untreated control samples were measured in 12 wells. Data was evaluated as % control (treated sample/untreated sample x 100) for each oligonucleotide.

6. <u>Inhibition of HCV RNA Expression in Stably Transfected Cells</u>

Cells were transfected with pcHCVneo, and cells stably expressing HCV C protein were selected by Western blot using a rabbit polyclonal antiserum specific for HCV protein (Hoffmann-La Roche, Basel, Switzerland). Cells also expressed HCV RNA as detected by ribonuclease protection assay using probes specific for the 5' UTR and HCV C protein coding sequence.

A ribonuclease protection assay was used to measure HCV RNA in HepG2 cells stably transfected with pcHCVneo. HCV specific riboprobes were prepared which included HCV sequences 52 to 338 (probe 1) or 238 to 674 (probe 2). HepG2 HCV cells (1 x 10^6 cells)

were seeded into 100 mm dishes, incubated overnight, then treated with oligonucleotide in the presence of 10 μg/ml Lipofectin for 4 hours as described above. Cells were incubated overnight. Total RNA was isolated using Trizol (Gibco/BRL, Gaithersburg, MD) according to the manufacturer's instructions.

Ribonuclease protection assays were performed using 10 μg of RNA. RNA was hybridized with radiolabelled probe overnight and then digested with single-strand specific RNases A and T1 (RPAII kit, Ambion, Austin, TX) according to the manufacturer's instructions. Ribonuclease digestion products were separated on a 6% polyacrylamide/urea gel. The gel was dried and exposed to x-ray film overnight. Molecular weights were estimated by comparison to RNA standards electrophoresed on the same gel (Ambion, Austin, TX). In addition, amounts of RNA were quantitated on a phosphorimager (BioRad GS250, Hercules, CA).

7. Inhibition of Protein Expression in SFV/HCV Infected Cells

HCV bases 1-2545 were used to generate a recombinant virus with Semliki Forest virus (SFV/HCV) (Gibco/BRL, Gaithersburg, MD). HCV sequences were subcloned from vv1-2545 (Hoffmann-La Roche, Basel, Switzerland) into pSFV1. SFV/HCV sequences were transcribed in vitro using SP6 RNA polymerase. RNA was also transcribed from pSFV2-Helper (Gibco/BRL, Gaithersburg, MD) which provided SFV structural proteins to the recombinant virus. The two RNAs were co-transfected into BHK21 cells (ATCC Ac. No. CCL 10, American Type Culture Collection, Rockville, MD), according to the manufacturer's instructions (SFV Gene Expression System, Gibco/BRL, Gaithersburg, MD.) to generate the recombinant virus. Supernatant was removed from the cultures 48 hours post-transfection and used as a virus stock for subsequent experiments. pSFV2-Helper produces a structural protein (p62) containing an eight base mutation, converting three arginines to non-basic amino acids. This modification renders the recombinant virus non-infectious unless the p62 protein is first digested with chymotrypsin (Gibco/BRL, Gaithersburg, MD). Recombinant virus required chymotrypsin activation before infection.

HepG2 cells (10^5 cells/well in a 6 well dish) were pretreated for 4 hours with different concentrations of oligonucleotide in the presence of 10 µg/ml Lipofectin in Optimem. Oligonucleotide was then removed, and cells were infected with chymotrypsin activated SFV/HCV (diluted 1/100 in PBS with Ca^{2+}, Mg^{2+}) for 1 hour at 37°C. The inoculum was removed, oligonucleotide in Optimum was added to cells, and cells were incubated overnight at 37°C. Cells were then lysed, protein was quantitated and equal amounts of protein were electrophoresed on an SDS/polyacrylamide gel. Protein was detected by Western blotting. The blots were scanned with a flat bed scanner (Umax Data Systems Inc., Hsinchu, Taiwan, ROC) and quantitated with densitometric software (Scan Analysis Biosoft, Ferguson, MO).

Alternatively, SFV/HCV virus stocks were prepared as described previously. SFV/HCV inhibition was measured as described previously except that, in some experiments, HepG2 cells were infected with SFV/HCV virus for one hour at 37°C, virus incolum was removed, and then oligonucleotide was added in the presence of lipofectin. In some experiments, cells were not incubated in the presence of oligonucleotide before infection. That oligonucleotides of the invention inhibited HCV C protein production in this assay system is shown below in Table 8.

Table 8

Sequence	SEQ ID No.	Backbone	≥40% Inhibition at 2, 0.4µM
HCV1	28	PS	yes
HCV3	35	PS	yes
HCV1 (EG4-20)	28	0x5 2'OMe PS	yes
HCV1 (EG4-23)	28	5x5 2'OMe PS	yes
HCV1	28	6x6 2'OMe PS	yes
HCV1	28	3x11 2'OMe PS	yes
HCV1 (EG4-29)	28	0x5 2'OMe PS	yes
HCV8	9	PS	yes
HCV28	30	PS	yes
HCV45	23	PS	yes

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: F.HOFFMANN-LA ROCHE AG
(B) STREET: Grenzacherstrasse 124
(C) CITY: Basle
(D) STATE: BS
(E) COUNTRY: Switzerland
(F) POSTAL CODE (ZIP): CH-4070
(G) TELEPHONE: 061 - 688 39 43
(H) TELEFAX: 061 - 688 13 95
(I) TELEX: 962292/965542 hlr ch

(A) NAME: Hybridon, Inc
(B) STREET: One Innovation Drive
(C) CITY: Worcester
(D) STATE: MA
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 01605
(G) TELEPHONE: 508/752-7000
(H) TELEFAX:508/752-7001

(ii) TITLE OF INVENTION: OLIGONUCLEOTIDES SPECIFIC FOR HEPATITIS C VIRUS

(iii) NUMBER OF SEQUENCES: 77

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: F.HOFFMANN-LA ROCHE AG
(B) STREET: Grenzacherstrasse 124
(C) CITY: Basle
(D) STATE: BS
(E) COUNTRY: Switzerland
(F) ZIP: CH-4070

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: System 7.1 (Macintosh)
(D) SOFTWARE: Word 5.1

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/471,968
(B) FILING DATE: 06.06.1995

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGTGCACGGT CTACGAGACC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATGGTGCAC GGTCTACGAG 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCTCATGGTG CACGGTCTAC 20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTGCTCATGG TGCACGGTCT 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGTGCTCATG GTGCACGGTC 20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
TTCGTGCTCA TGGTGCACGG 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGATTCGTGC TCATGGTGCA 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTAGGATTCG TGCTCATGGT 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGTTTAGGAT TCGTGCTCAT 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGGTTTAG GATTCGTGCT 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTTGAGGTT TAGGATTCGT 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTCTTTGAGG TTTAGGATTC 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TACGTTTGGT TTTTCTTTGA 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTTGGTGTTA CGTTTGGTTT 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCACGACACT CATACTAACG 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGCTGCACGA CACTCATACT 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGGAGGCTGC ACGACACTCA 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCCTGGAGG CTGCACGACA 20

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGGGTCCTG GAGGCTGCAC 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAGGGGGGGT CCTGGAGGCT 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCGGGAGGGG GGGTCCTGGA 20

(2) INFOPMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGCTCTCCCG GGAGGGGGGG 20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCACTATGGC TCTCCCGGGA 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AACACTACTC GGCTAGCAGT 20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

ACCCAACACT ACTCGGCTAG 20

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGACCCAACA CTACTCGGCT 20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGCGACCCAA CACTACTCGG 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TTCGCGACCC AACACTACTC 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CTTTCGCGAC CCAACACTAC 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCCTTTCGCG ACCCAACACT 20

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AGGCCTTTCG CGACCCAACA 20

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAAGGCCTTT CGCGACCCAA 20

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CACAAGGCCT TTCGCGACCC 20

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ACCACAAGGC CTTTCGCGAC 20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AGTACCACAA GGCCTTTCGC 20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTCTACGAGA CCTCCCGGG 19

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GCACGGTCTA CGAGACCTCC 20

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGGUCCUGG AGNNNNNN 18

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GGGGUCCUGG AGGACCGG 18

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GACCGGGGGG UCCUGGAG 18

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGGGUCCUGG AGAGGATT 18

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AGGATTGGGG UCCUGGAG 18

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGGGUCCUGG AGCATGGT 18

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CATGGTGGGG UCCUGGAG 18

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GGGGUCCUGG AGCGTGCT 18

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CGTGCTGGGG UCCUGGAG 18

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGGGUCCUGG AG 12

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGGGTCCTGG AGCATGGTGC ACGG 24

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGGUCCUGG AGGGTGCA 18

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGTGCAGGGG UCCUGGAG 18

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGGGUCCUGG AGGCTCAT 18

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GCTCATGGGG TCCTGGAG 18

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GGGGUCCUGG AGATTCGT 18

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

ATTCGTGGGG UCCUGGAG 18

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GGGGTCCTGG AGAGGATTCG TGCT 24

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGGGUCCUGG AGCGTGCTCA TGGT 24

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CATGGTGCAC GGGGGGTCCT GGAG 24

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

TGGATTCGTG CAGGGGTCCT GGAG 24

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CGTGCTCATG GTGGGGTCCT GGAG 24

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGGGTCCTGG AGATTCGTGC TCAT 24

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATTCGTGCTC ATGGGGTCCT GGAG 24

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGGGTCCTGG AGTGGTGCAC GGTC 24

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TGGTGCACGG TCGGGGTCCT GGAG 24

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGGGUCCUGG AGGCTCATGG TGCA 24

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GCTCATGGTG CAGGGGUCCU GGAG 24

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GGGGTCCTGG AGGCACGGTC TACG 24

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA/RNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GGGGUCCUGG AGNNNNNNNN NNNN 24

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TTCGCGACCC AACACTACTC GGCTAGCA 28

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:
CATGGCTAGA CGCTTTCTGC 20

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CTCGCGGGGG CACGCCCAAA 20

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

TGAGCGGGTT GATCCAAGAA 20

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GATCCAAGAA AGGACCCGGT 20

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GGCTAGCAGT CTCGCGGGGG 20

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GCCTTTCGCG ACCCAACACT ACTCGGCT Seq ID #12 28

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CTTTCGCGAC CCAACACTAC TCGG 24

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CGCGACCCAA CACTAC 16

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GGGGCACTCG CAAGCACCCT 20

CLAIMS

1. A synthetic oligonucleotide complementary to a portion of the 5' untranslated region of hepatitis C virus and having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 2, 5, 6, 7, 8, 14, 15, 16, 23, 24, 26, 27, 28, 29, 31, 33, 36, 37, 47, 68, 69, 70, 71, 72, 73, 74, 75, 76, and 77 as set forth in Table 1F or selected from the group consisting of SEQ ID Nos. 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108. 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, and 133 as set forth in Table 1A and Table 1B.

2. A synthetic oligonucleotide comprising a sequence complementary to at least two non-contiguous regions of an HCV messenger or genomic RNA.

3. An oligonucleotide according to claim 2 wherein the sequence is complementary to three non-contiguous regions.

4. A synthetic oligonucleotide according to claim 2 or 3 wherein one of the non-contiguous regions is the 5' untranslated region.

5. An oligonucleotide according to claim 2 having about 18 to about 24 nucleotides.

6. An oligonucleotide according to claim 2 wherein one portion of the oligonucleotide has the sequence GGGGUCCUGGAG (SEQ ID NO:47) or has the sequence CAACACUACUCG.

7. A synthetic oligonucleotide according to any one of claims 1-6 which is modified.

8. An oligonucleotide according to claim 7 wherein the modification comprises at least one internucleotide linkage selected from the group consisting of alkylphosphonate, phosphorothioate, phosphorodithioate, alkylphosphonothioate, phosphoramidate, carbamate, carbonate,

phosphate triester, acetamidate, carboxymethyl ester, and combinations thereof.

9. An oligonucleotide according to claim 8 comprising at least one phosphorothioate internucleotide linkage.

10. An oligonucleotide according to claim 8 wherein the internucleotide linkages in the oligonucleotide are phosphorothioate internucleotide linkages.

11. An oligonucleotide according to claim 7 which comprises at least one deoxyribonucleotide.

12. An oligonucleotide according to claim 7 which comprises at least one ribonucleotide.

13. An oligonucleotide according to claim 11 which additionally comprises at least one ribonucleotide.

14. An oligonucleotide according to claim 13 wherein an oligodeoxyribonucleotide region is interposed between two oligoribonucleotide regions, or the inverted configuration thereof.

15. An oligonucleotide according to any one of claims 12-14 wherein the ribonucleotide is a 2'-O-methyl ribonucleotide.

16. An oligonucleotide according to claim 13 which comprises at least one 2'-O-methyl ribonucleotide at the 3'-end of the oligonucleotide.

17. An oligonucleotide according to claim 16 which further comprises at least one 2'-O-methyl ribonucleotide at the 5'-end of the oligonucleotide.

18. An oligonucleotide according to claim 13 having a nucleotide sequence, selected from the group consisting of SEQ ID NOS: 119-130, as set forth in Table 1A.

19. An oligonucleotide according to claim 2 comprising a sequence selected from the group consisting of SEQ ID NOS: 38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, and 67, as set forth in Table 2.

20. An oligonucleotide according to claim 2 comprising a sequence selected from the group consisting of SEQ ID NOS: 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146 and 147, as set forth in Table 1C.

21. An oligonucleotide according to claim 3 comprising a sequence selected from the group consisting of SEQ ID NOS: 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, and 158, as set forth in Table 1D.

22. An oligonucleotide according to claim 7 which oligonucleotide is self stabilized by a loop.

23. An oligonucleotide according to claim 21 having a sequence selected from the group consisting of SEQ ID NOS: 131, 132 and 133 as set forth in Table 1B.

24. An oligonucleotide according to claim 7 wherein the modification is selected from the group consisting of a nicked dumbell, a closed dumbell, 2', 3' and/or 5' caps, additions to the molecule at the internucleotide phosphate linkage, oxidation, oxidation/reduction, and oxidation/reductive amination, including combination thereof.

25. An oligonucleotide according to claim 7 wherein at least one nucleoside is substituted by inosine or wherein at least one deoxycytosine is substituted by 5-methyl deoxycytosine.

26. An oligonucleotide according to claim 25 wherein the oligonucleotide is selected from the group consisting of SEQ ID NOS: 117 (HCV-242, HCV 243, HCV-244) and 118 (HCV-245) as set forth in Table 1A.

27. An oligonucleotide according to claim 7 wherein the contiguous or non-contiguous oligonucleotide is modified by incorporating at least one additional triplex forming strand.

28. An oligonucleotide according to claim 27 having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, and 172 as set forth in Table 1E.

29. A pharmaceutical composition comprising at least one oligonucleotide according to any one of claims 1-28 and a pharmaceutically acceptable carrier.

30. A pharmaceutical composition comprising at least two different oligonucleotides according to any one of claims 1-28.

31. An oligonucleotide according to any one of claims 1-28, for use as a therapeutically active compound, especially for use in the control or prevention of hepatitis C virus infection or replication.

32. Use of an oligonucleotide according to any one of claims 1-28 for inhibiting hepatitis C virus replication in a cell or for treating hepatits C virus infection.

33. A method of detecting the presence of HCV in a sample, comprising the steps of:

(a) contacting the sample with a synthetic oligonucleotide according to claim 1 or claim 2; and

(b) detecting the hybridization of the oligonucleotide to the nucleic acid.

34. A kit for the detection of HCV in a sample comprising:

(a) a synthetic oligonucleotide according to claim 1 or claim 2; and

(b) means for detecting the oligonucleotide hybridized with the nucleic acid.

35. The new oligonucleotides, formulations and methods as herein described.

***

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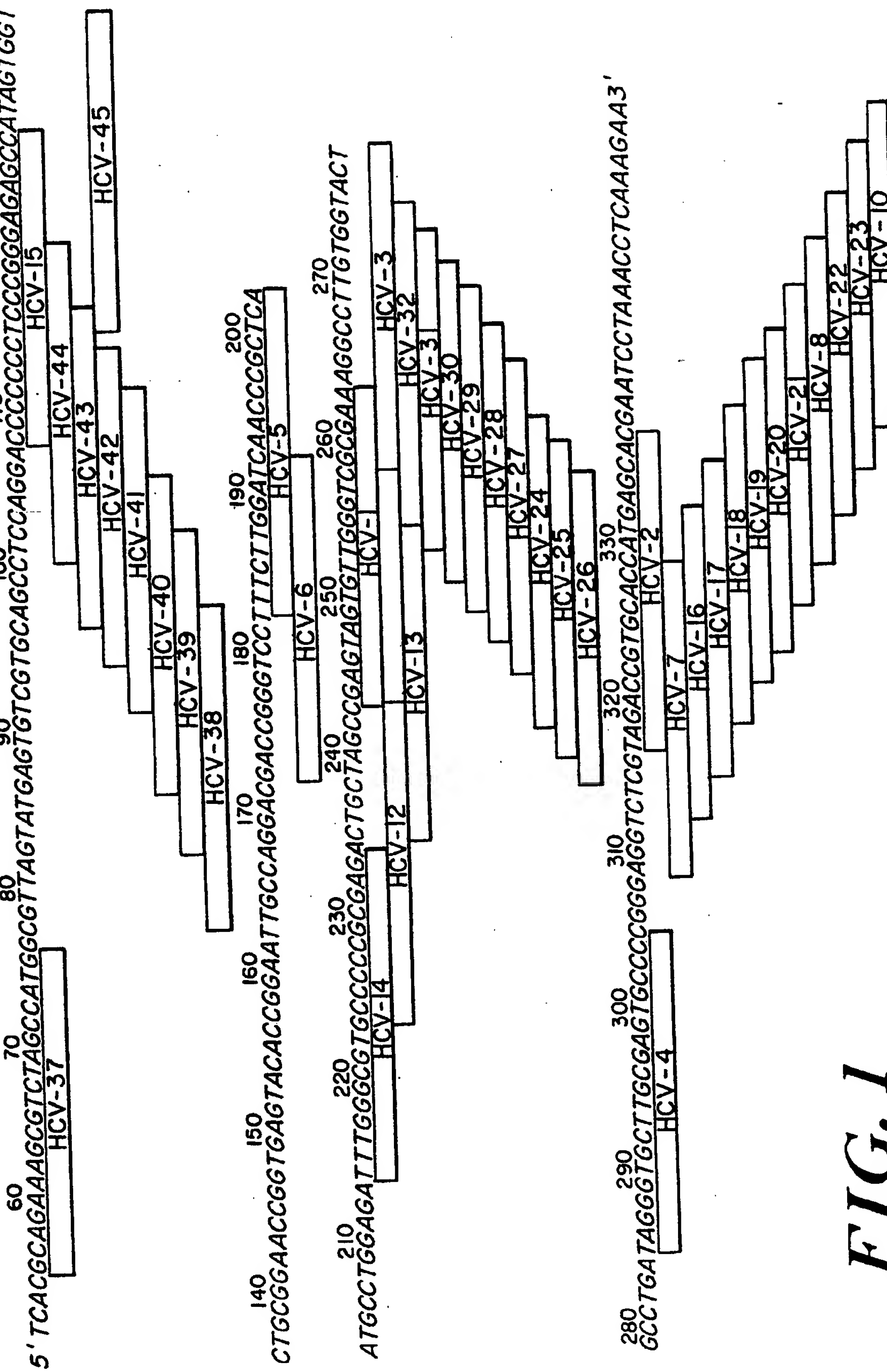
5' TCACGCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGTGGT
CTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATCAACCCGCTCA
ATGCCTGGAGATTTGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGTACT
GCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAA 3'
60 70 80 90 100 110 120 130
140 150 160 170 180 190 200
210 220 230 240 250 260 270
280 290 300 310 320 330
HCV-37 HCV-15 HCV-44 HCV-43 HCV-42 HCV-41 HCV-40 HCV-39 HCV-38 HCV-45
HCV-5 HCV-6
HCV-14 HCV-12 HCV-1 HCV-13 HCV-3 HCV-32 HCV-31 HCV-30 HCV-29 HCV-28 HCV-27 HCV-24 HCV-25 HCV-26
HCV-4 HCV-2 HCV-7 HCV-16 HCV-17 HCV-18 HCV-19 HCV-20 HCV-21 HCV-8 HCV-22 HCV-23 HCV-10

FIG. 1

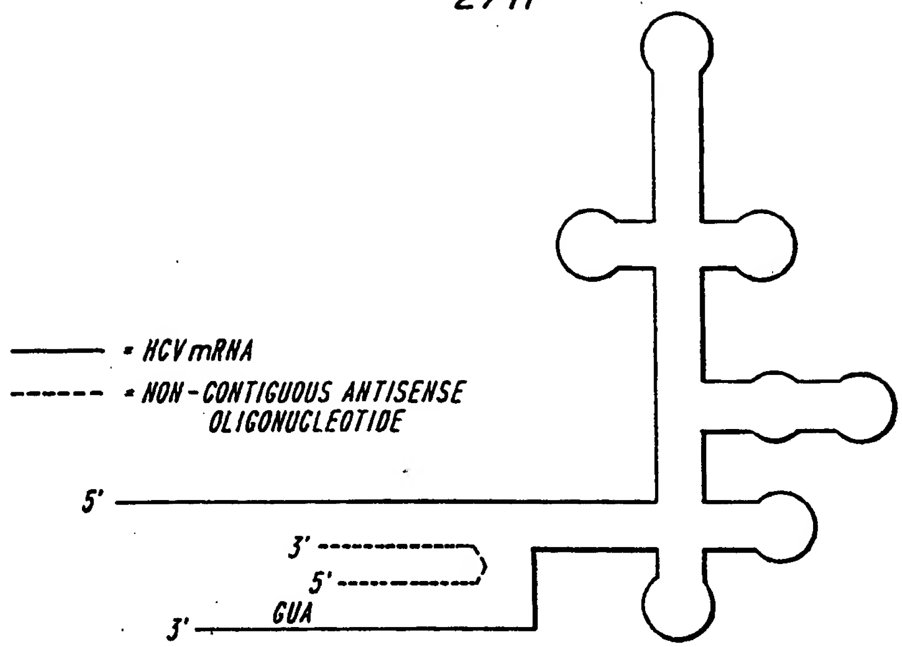
= HCV mRNA
= NON-CONTIGUOUS ANTISENSE OLIGONUCLEOTIDE
5'
3'
5'
GUA
3'

FIG. 2A

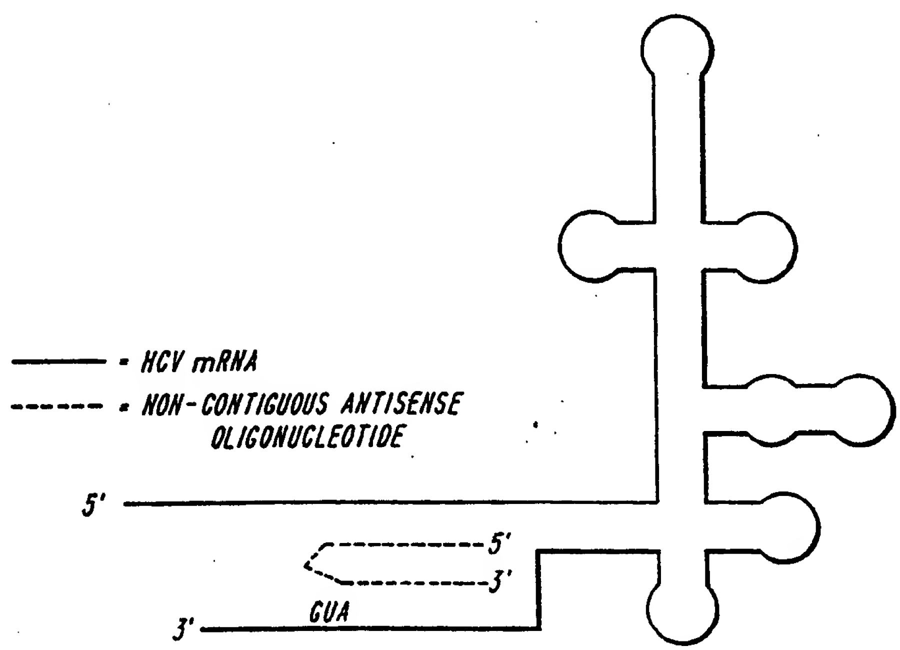
= HCV mRNA
= NON-CONTIGUOUS ANTISENSE OLIGONUCLEOTIDE
5'
5'
3'
GUA
3'

FIG. 2B

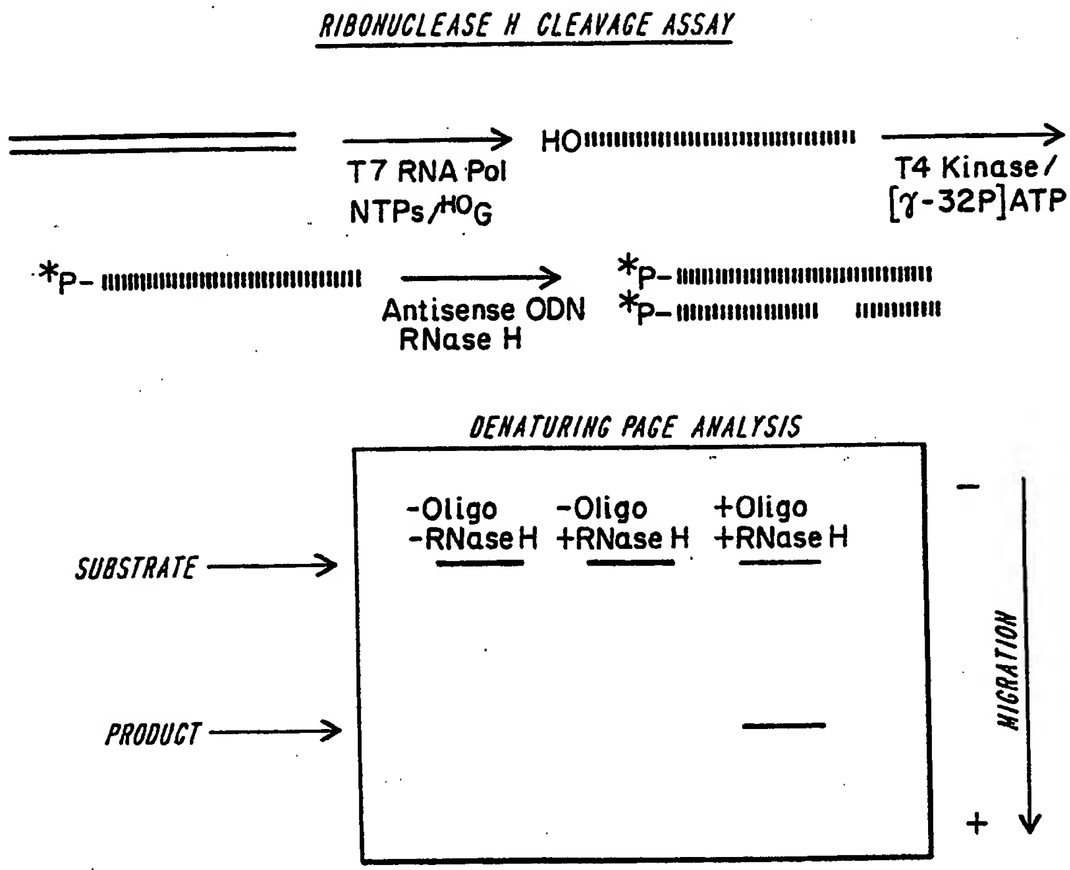
RIBONUCLEASE H CLEAVAGE ASSAY
HO
T7 RNA Pol
NTPs/HOG
T4 Kinase/
[γ-32P]ATP
*P-
Antisense ODN
RNase H
*P-
*P-
DENATURING PAGE ANALYSIS
-Oligo
-RNase H
-Oligo
+RNase H
+Oligo
+RNase H
SUBSTRATE
PRODUCT
−
+
MIGRATION

FIG. 3

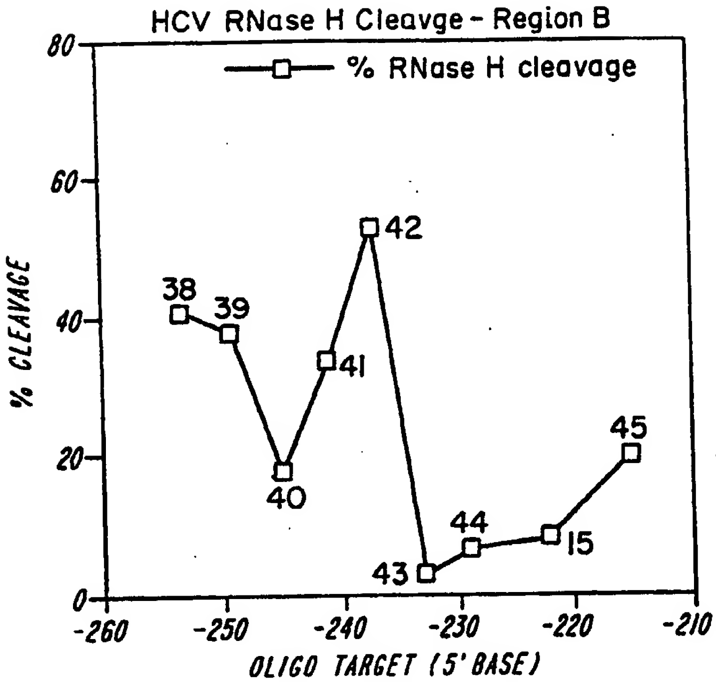
HCV RNase H Cleavge - Region B
% RNase H cleavage
% CLEAVAGE
OLIGO TARGET (5' BASE)
0
20
40
60
80
-260
-250
-240
-230
-220
-210
38
39
40
41
42
43
44
15
45

FIG. 4A

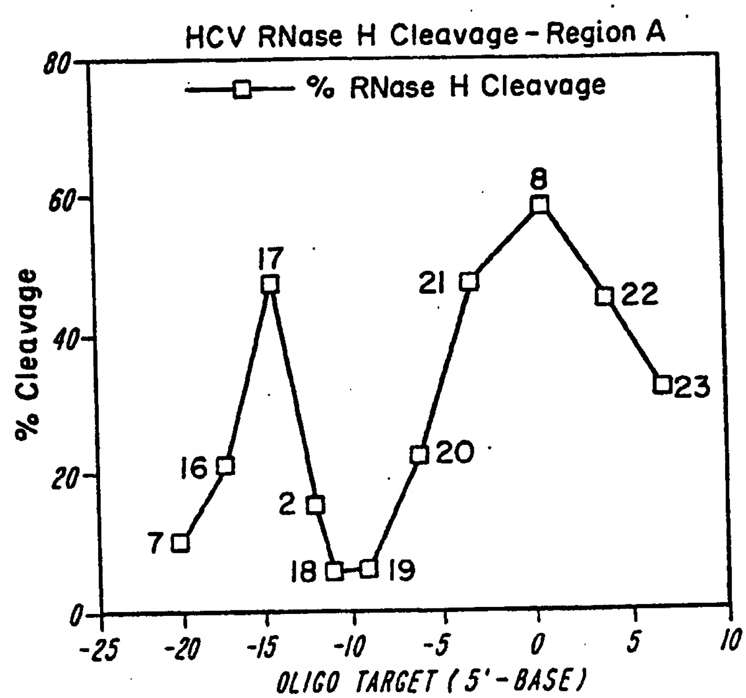
HCV RNase H Cleavage - Region A
% RNase H Cleavage
% Cleavage
OLIGO TARGET (5'-BASE)
0
20
40
60
80
-25
-20
-15
-10
-5
0
5
10
7
16
17
2
18
19
20
21
8
22
23

FIG. 4B

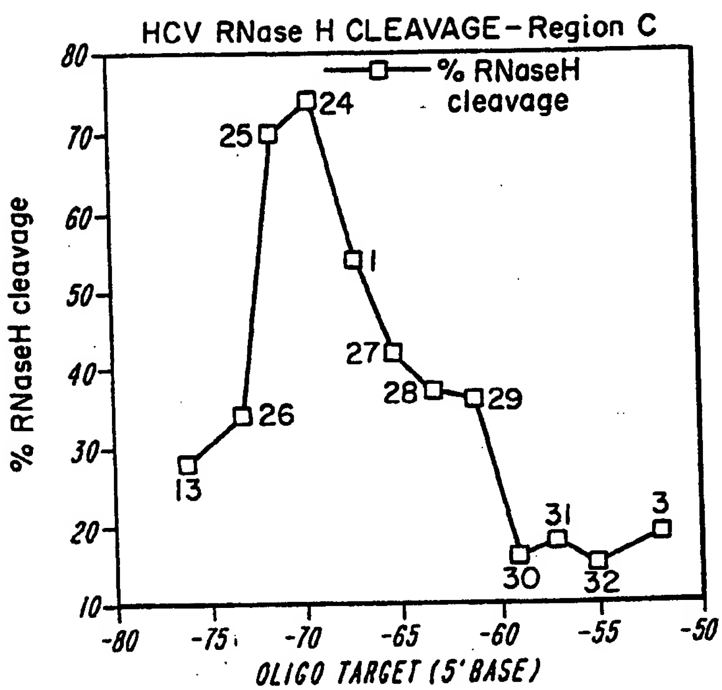
HCV RNase H CLEAVAGE-Region C
% RNaseH cleavage
% RNaseH cleavage
OLIGO TARGET (5' BASE)
80
70
60
50
40
30
20
10
-80
-75
-70
-65
-60
-55
-50
13
26
25
24
1
27
28
29
30
31
32
3

FIG. 4C

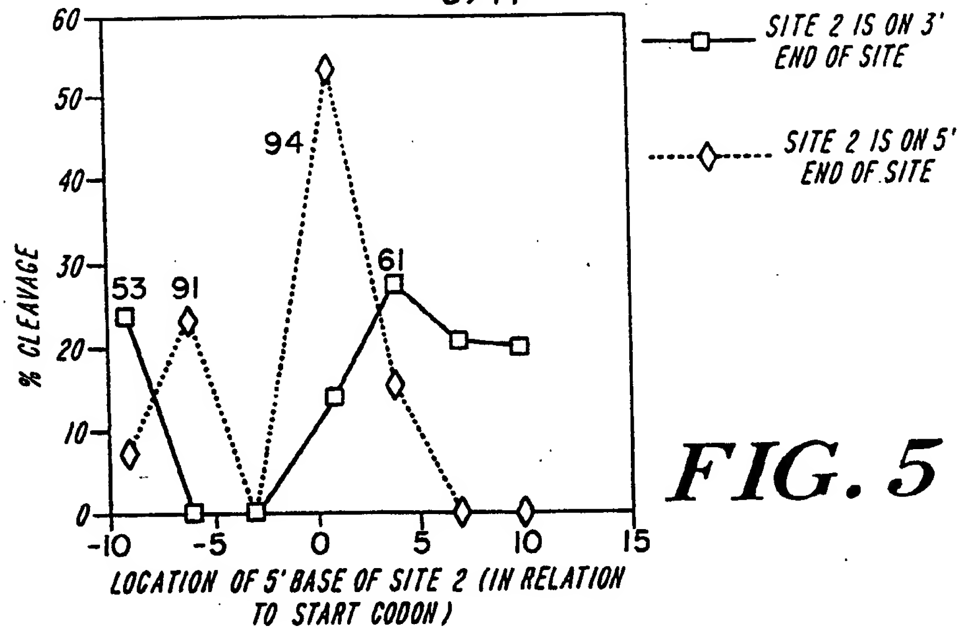
60
50
40
30
20
10
0
% CLEAVAGE
53
91
94
61
-10
-5
0
5
10
15
LOCATION OF 5' BASE OF SITE 2 (IN RELATION TO START CODON)
SITE 2 IS ON 3' END OF SITE
SITE 2 IS ON 5' END OF SITE

FIG. 5

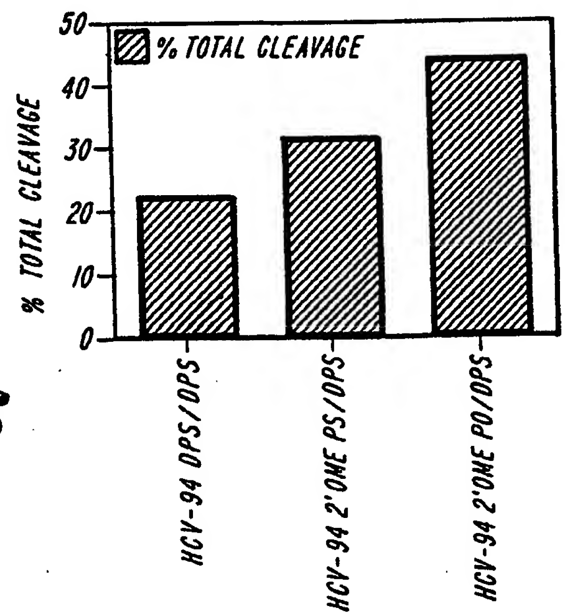
50
40
30
20
10
0
% TOTAL CLEAVAGE
% TOTAL CLEAVAGE
HCV-94 OPS/OPS
HCV-94 2'OME PS/OPS
HCV-94 2'OME PO/OPS

FIG. 6

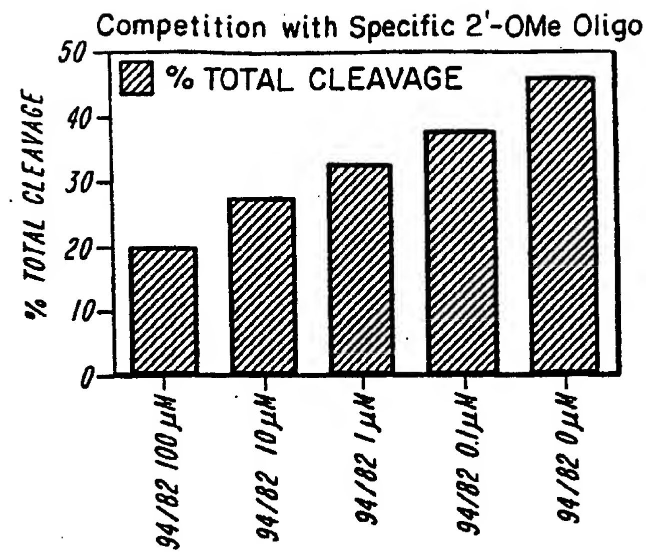
Competition with Specific 2'-OMe Oligo
% TOTAL CLEAVAGE
% TOTAL CLEAVAGE
50
40
30
20
10
0
94/82 100μM
94/82 10μM
94/82 1μM
94/82 0.1μM
94/82 0μM

FIG. 7

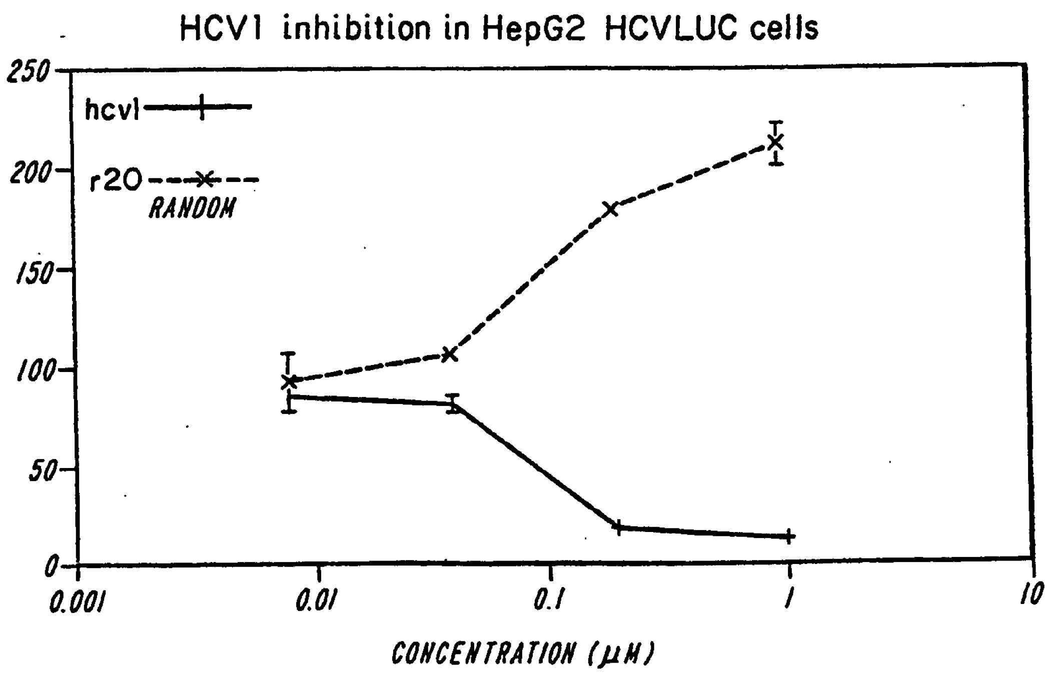
HCV1 inhibition in HepG2 HCVLUC cells
hcvl
r20
RANDOM
250
200
150
100
50
0
0.001
0.01
0.1
1
10
CONCENTRATION (μM)

FIG. 9

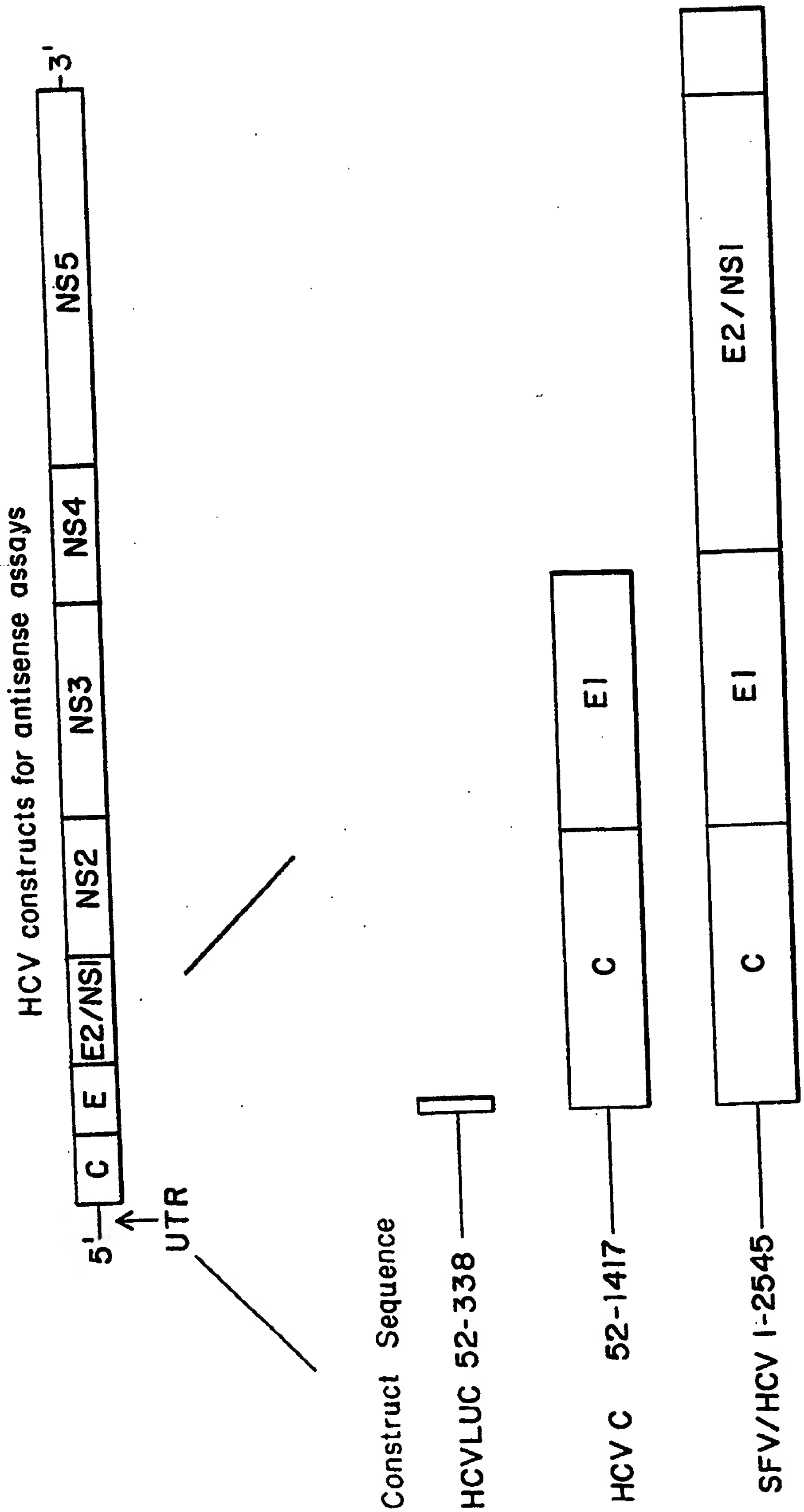
HCV constructs for antisense assays
5'
C
E
E2/NSI
NS2
NS3
NS4
NS5
3'
UTR
Construct Sequence
HCVLUC 52-338
HCV C 52-1417
C
EI
SFV/HCV 1-2545
C
EI
E2/NSI

FIG. 8

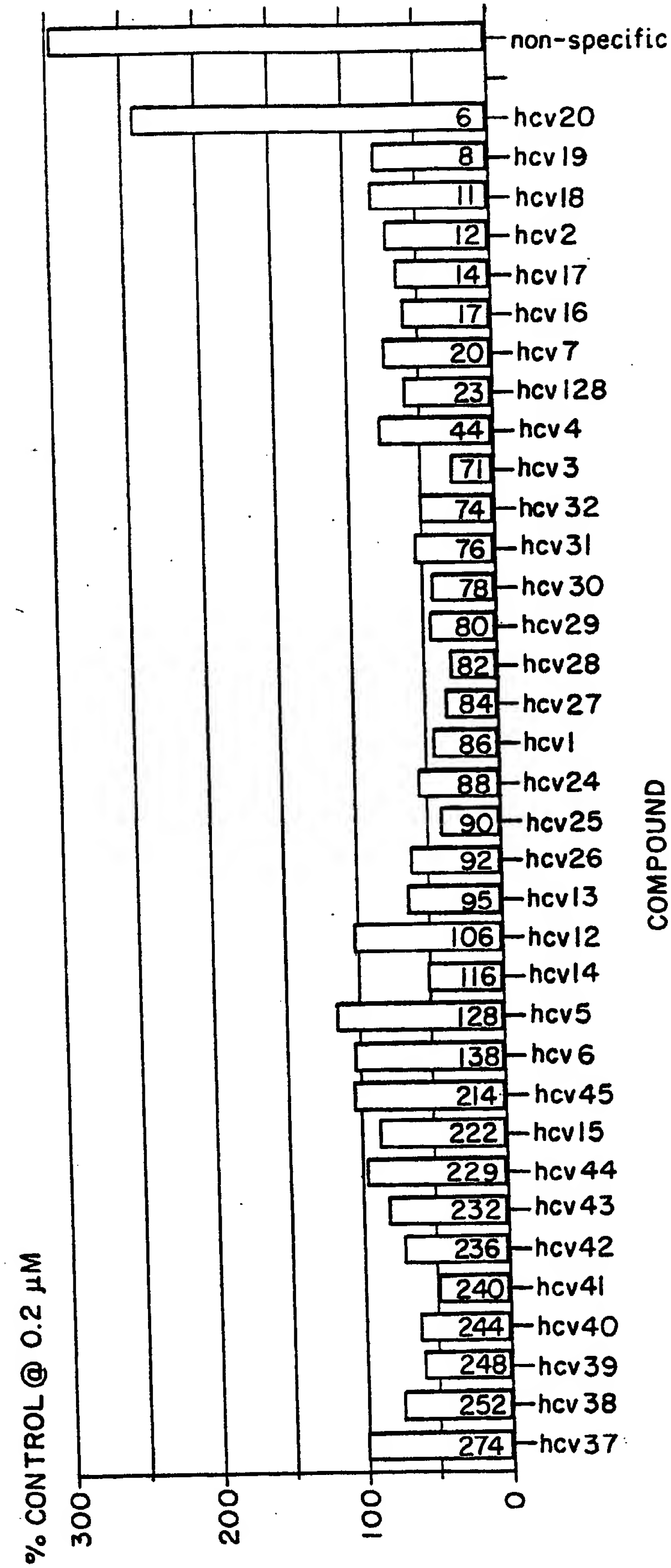
% CONTROL @ 0.2 µM
300
200
100
0
COMPOUND
non-specific
6 hcv20
8 hcv19
11 hcv18
12 hcv2
14 hcv17
17 hcv16
20 hcv7
23 hcv128
44 hcv4
71 hcv3
74 hcv32
76 hcv31
78 hcv30
80 hcv29
82 hcv28
84 hcv27
86 hcv1
88 hcv24
90 hcv25
92 hcv26
95 hcv13
106 hcv12
116 hcv14
128 hcv5
138 hcv6
214 hcv45
222 hcv15
229 hcv44
232 hcv43
236 hcv42
240 hcv41
244 hcv40
248 hcv39
252 hcv38
274 hcv37

FIG. 10

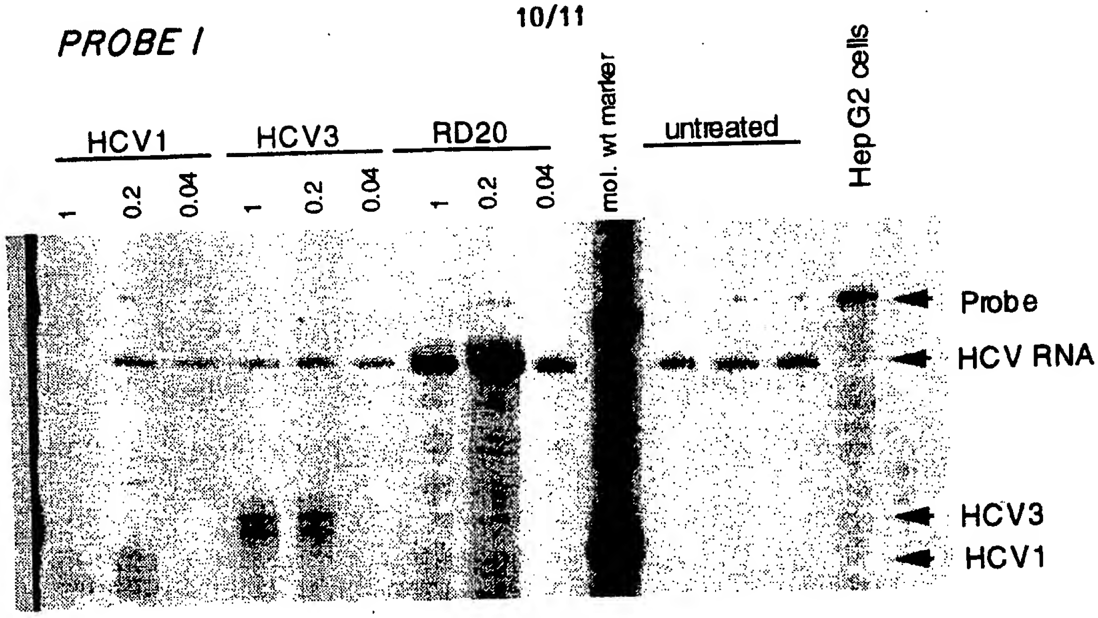

FIG. 11A

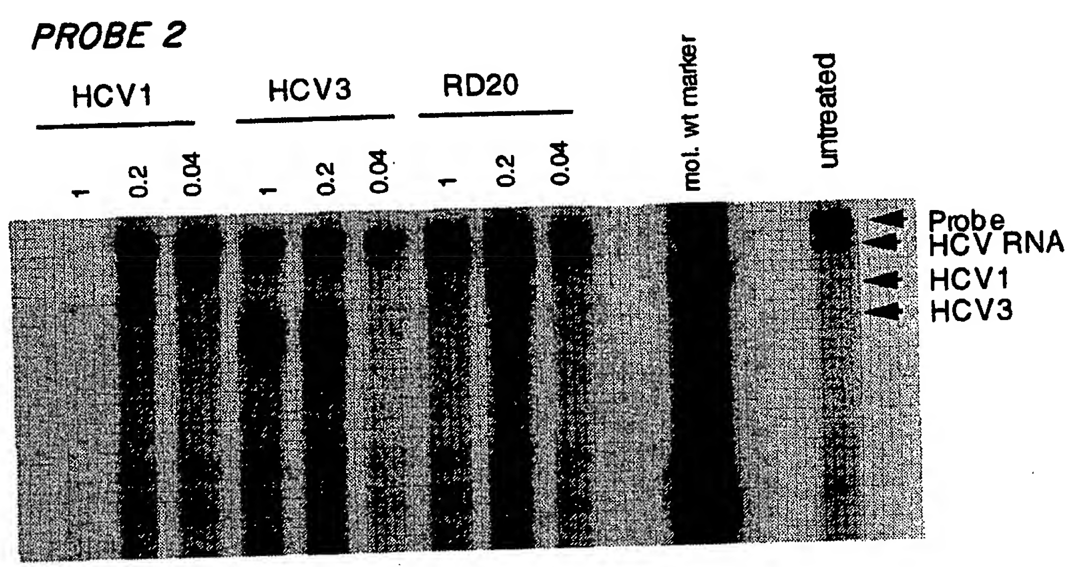

FIG. 11B

C. HCV RNA and RPA probes

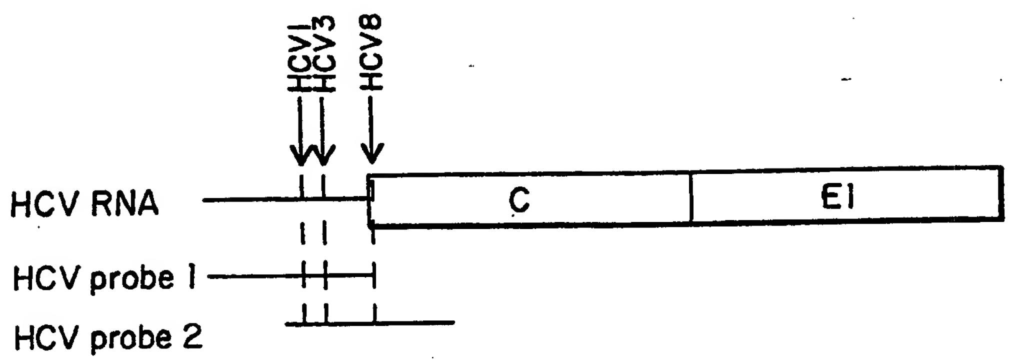

FIG. 11C